PB-227 560

PROCEEDINGS OF THE SHELLFISH SANITATION WORKSHOP, (4TH), NOVEMBER 28-30, 1961

Eugene T. Jensen

Public Health Service
Washington, D.C.

1961

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PROCEEDINGS

SHELLFISH SANITATION WORKSHOP

November 28-30, 1961

Edited by
Eugene T. Jensen
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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service

Division of Environmental Engineering and Food Protection

Shellfish Sanitation Branch

Washington 25, D.C.

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The 1961 Shellfish Sanitation Workshop was arranged by the Public Health Service in accord with the recommendations of the 1958 Workshop. The objectives of the Workshop were: (1) discuss current administrative and technical problems; (2) review numerous proposals for changes in the Manual of Recommended Practice for Sanitary Control of the Shellfish Industry; (3) review research findings related to the Cooperative Program; and (4) consider the present advisability of establishing a bacteriological standard for shucked Eastern oysters at the market level.

Invitations to attend the Workshop were extended to all State and Territorial Health Departments, Marine Fishery Departments in the coastal States, Health Departments in major cities, interested Federal agencies, Governments of Canada and Japan, and the shellfish industry trade organizations. The Workshop was attended by one hundred forty-eight persons representing seventy-four agencies or organizations.

The following proceedings are based on the prepared papers and a transcript. The proceedings have been reviewed for accuracy by the various speakers.

Our appreciation is extended to the many persons whose efforts contributed to the success of this undertaking.

Eugene T. Jensen, Chief
Shellfish Sanitation Branch
Division of Environmental Engineering
and Food Protection

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
PUBLIC HEALTH SERVICE

AGENDA

FOURTH NATIONAL SHELLFISH SANITATION WORKSHOP

November 28-30, 1961
Room 5051 -- HEW Building
3rd and Independence Ave., S.W.
Washington, D. C.

Tuesday morning, November 28, 1961

CHAIRMAN: John D. Faulkner, Chief
Milk and Food Program
Division of Environmental Engineering
and Food Protection

9:30 Opening Remarks

9:45 A report on the status of the Cooperative State-PHS-Industry Program for the Certification of Interstate Shellfish Shippers
. . . . . E. T. Jensen, Public Health Service

10:30 Break

10:45 A general discussion of necessary changes in the review draft of the manual for PHS appraisal of State shellfish sanitation programs
. . . . . W. A. Felsing, Public Health Service
. . . . . E. T. Jensen, Public Health Service

12:00 Lunch

Tuesday afternoon, November 28, 1961

CHAIRMAN: Dr. Arthur F. Novak
Louisiana State University

1:15 A general discussion of proposed changes in Parts I and II of the shellfish sanitation manuals, including a final decision on each change to be made
. . . . . L. R. Lunsford, Public Health Service
. . . . . E. T. Jensen, Public Health Service

2:45 Break

3:00 A general discussion of problems related to the sanitary control of shellfish imports

Department of State viewpoint
. . . . . (speaker to be announced)
Public Health Service viewpoint
. . . . . John D. Faulkner, Public Health Service

4:00 Report on hot-dip (shock) method of opening oysters
. . . . . Somers B. Pringle
South Carolina State Board of Health

4:30 A panel discussion of the need for sanitary controls in the scallop and crabmeat industries

. . . . . Ross E. Homans, Chief Inspector
Canada Department of Fisheries
. . . . . Frederick J. Wilbour
Massachusetts Division of Marine Fisheries

5:30 Adjourn

Tuesday evening, November 28, 1961

6:15 Get-acquainted dinner

Wednesday morning, November 29, 1961

CHAIRMAN: David H. Wallace, Director
Oyster Institute of North America

9:30 A review of results obtained in the collaborative study of the bacteriological quality of shucked Eastern oysters to determine if bacteriological standards are to be incorporated in the cooperative program and, if so, to formulate an administrative procedure for using the standard

. . . . . C. B. Kelly, Public Health Service

10:45 Break

11:00 Final review of construction guides for shellfish handling and packaging equipment

. . . . . L. R. Lunsford, Public Health Service

11:30 Discussion of the chromate test for oyster freshness and its use in oyster sanitation programs

. . . . . Dr. Mary Schafer, Public Health Service
. . . . . J. A. Hope, Virginia State Dept. of Health

12:15 Lunch

Wednesday afternoon, November 29, 1961

CHAIRMAN: Dr. George H. Hauser, Acting Director
Division of Food and Drug
Louisiana State Board of Health

1:30 A general review of the results obtained in the collaborative study of the sanitary significance of E. coli organisms in shellfish growing areas

. . . . . Dr. Joseph H. McCarthy, Chief
Lawrence Experiment Station
Massachusetts Dept. of Public Health
. . . . . Robert M. Brown, Chief
Bureau of Environmental Hygiene
Maryland State Dept. of Health
. . . . . J. A. Hope, Bacteriologist
Virginia State Dept. of Health
. . . . . Dr. Thomas S. Hosty, Director
Bureau of Laboratories
Alabama State Dept. of Public Health

. . . . . C. B. Kelly, Public Health Service
. . . . . Dr. A. D. Tennant, Canadian Dept.
National Health & Welfare
. . . . . Thomas P. Blair
Oregon State Board of Health
. . . . . Dr. A. Joel Kaplovsky, Director
Delaware State Water Pollution Commission
Delaware State Board of Health

3:15 Break

3:30 Resume discussions

4:30 A discussion of the sanitary significance of radionuclides in the marine environment from the standpoint of the shellfish sanitation program
. . . . . Dr. J. E. Campbell, Jr., Public Health Service
. . . . . Dr. Winston Decker, Public Health Service

5:30 Adjourn

Thursday morning, November 30, 1961

CHAIRMAN: John Glude, Chief
Branch of Shellfisheries
Bureau of Commercial Fisheries
Fish and Wildlife Service

9:30 A review of the use of the Conditionally Approved area concept
. . . . . E. T. Jensen, Public Health Service

10:30 Break

10:45 A discussion of the public-health significance of selected industrial wastes in shellfish growing areas
. . . . . Dr. Leonard Kurland, Public Health Service
. . . . . Earl McFarren, Public Health Service

12:00 Lunch

Thursday afternoon, November 30, 1961

CHAIRMAN: Robert M. Brown, Chief
Bureau of Environmental Hygiene
Maryland State Department of Health

1:15 A discussion of the possible public-health hazards involved in the use of chemicals for the control of shellfish predators

Fish and Wildlife Service research on the use of chemical control of shellfish predators
. . . . . Dr. Victor Loosanoff, Chief
U. S. Shellfisheries Laboratory
Milford, Connecticut

Food and Drug Administration viewpoint
. . . . . Robert S. Roe, Director
Bureau of Biological and Physical Sciences, FDA

Public Health Service viewpoint
. . . . .

Industry viewpoint

. . . . .

State viewpoint and discussion

. . . . .

3:15 Break

3:30 A panel discussion of the paralytic shellfish poison situation in the United States and Canada

. . . . . Dr. A. Prakash
Fisheries Research Board of Canada
. . . . . Phillip L. Goggins
Maine Dept. of Sea & Shore Fisheries
. . . . . A. K. Sparks
University of Washington
. . . . . Earl McFarren, Public Health Service

4:30 Adjourn

Tuesday Morning - November 28, 1961

Mr. David H. Wallace, Chairman

The Workshop convened in the Surgeon General's conference room in the Department of Health, Education, and Welfare building, Washington, D. C. at 9:30 a.m. After the invocation by Mr. William Woodfield, President, Oyster Growers and Dealers Association, the Chairman introduced the initial speaker, Dr. Robert J. Anderson, Chief, Bureau of State Services.

Dr. Anderson welcomed the group on behalf of the Surgeon General, Public Health Service, Department of Health, Education, and Welfare, to the Fourth Shellfish Sanitation Workshop. He pointed out that the attendance being over 120 persons, including representatives from Canada and, for the first time, observers from the Government of Japan, was evidence of the widespread interest in this program. He then discussed contemporary problems in shellfish sanitation, emphasizing the urgent need to identify and correct any deficiencies in the Cooperative Program; to review State programs, and to document evidence that State programs are, in fact, satisfactory for PHS endorsement. Dr. Anderson further pointed out the urgent need for a realistic appraisal of the financial and technical needs of State programs, recognizing that effective State programs require adequate financial and personnel resources. He also noted the need for an effective supporting research program, and discussed the recent actions of the Congress in authorizing the construction of Public Health Service shellfish sanitation research centers which would provide both research and technical consultation services to the program. In closing, Dr. Anderson reviewed the shellfish sanitation legislation which had been introduced in the past two sessions of Congress and emphasized the desirability of a legislative base for the existing Cooperative Program. (Dr. Anderson's complete statement is included as Appendix A of these Proceedings.)

Report on the Status of the Cooperative-State-PHS-Industry Program for the Certification of Interstate Shellfish Shippers:

The current status of the Cooperative Program for the Certification of Interstate Shellfish Shippers was reviewed by Mr. Eugene Jensen, Chief, Shellfish Sanitation Branch, Public Health Service. It was pointed out that experiences during the past year in which shellfish had been implicated in the transmission of infectious hepatitis demonstrated the need for a critical program review to identify and correct weaknesses in the existing certification procedures.

The problems of disposal of industrial and municipal wastes, and the impact of the pollution problem on the shellfish sanitation program was discussed. It was suggested that the problem of preventing pollution of shellfish areas could not be met entirely through the construction of sewage treatment plants. (See Appendix B, "The Application of the 'Conditionally Approved' Shellfish Growing Area Concept".) Mr. Jensen also discussed the dependency of the Cooperative Program on adequate research and noted that authority and funds had been obtained for the construction of two new PHS shellfish sanitation research centers to supplement the existing laboratory at Purdy, Washington. He noted that it was the intention of the PHS to operate these facilities along administrative lines similiar to those which had been used at Purdy; i.e., in addition to primary research, the centers would provide technical consultation on the problems of unusual technical complexities or magnitude.

It was pointed out by Mr. Jensen that technical literature of shellfish sanitation appears in many different journals and special publications, and that it is virtually impossible for the professional worker to keep informed of the many technical, administrative, and legislative developments at both State and Federal level. He suggested that considerations be given to the establishment of a PHS shellfish sanitation technical newsletter which would go to all people interested in shellfish sanitation.

The need for critical reappraisal of the States' operating programs and the relationships of these appraisals to the administration of the National Program was also reviewed. Mr. Jensen indicated that States would be asked for information on current levels of expenditures on shellfish sanitation, and for their estimates of programs that would be necessary to assure complete compliance with the standards of the Cooperative Program.

Mr. Jensen also noted that the PHS had recently developed a shellfish sanitation exhibit which could be made available to interested organizations for use at meetings.

Review of Proposed Manual for Public Health Service Appraisal of State Shellfish Sanitation Programs:

Mr. Jensen explained that when the Cooperative Program was established in 1925, it was agreed that States, PHS, and the industry would each have specific administrative responsibilities. Under this agreement, the PHS accepted responsibility for research, development of uniform standards, and the evaluation of State programs to determine the compliance with standards.

The Service has never developed explicit instructions for program evaluation or established minimum standards for endorsement. Consequently, neither the States nor the PHS has known exactly how the evaluations would be made. Initially, this PHS responsibility for evaluation of State programs was met through the preparation of brief narrative reports. In 1946 or 1947 a PHS policy guide for the shellfish program was included in the Division of Sanitation Manual of Operations. Although the adoption of this system was a forward step, it was defective in many respects. At the 1958 Shellfish Sanitation Workshop, it was suggested that the PHS develop a more objective review process and minimum standards for endorsement. On the basis of previous experience in this field, an initial draft was developed in 1960 and sent to the Regional Consultants for consideration. A second review draft was prepared and distributed in 1961 to the State and Federal agencies and to the shellfish industry for review. Mr. Jensen noted that the proposed manual has been used by the regional shellfish sanitation consultants in several instances and also by the PHS in the 1961 study of shellfish control measures in Hiroshima, Japan. He reported that it was also discussed at meetings with State agencies in Boston, Trenton, and Richmond. He emphasized that Part III contained no new program requirements, but was essentially (1) an inspection report for use with the existing Parts I and II of the manual and, (2) a PHS operating manual.

To assist the Workshop in reviewing the proposed manual, the suggested changes in the February 1, 1961 review draft had been tabulated and referenced. Each such change was then discussed.

1. Need for Formal Request From States to Participate in the Cooperative Program:

Mr. Udell, New York Conservation Department, indicated that it was their position that each State should make an official request to participate in the program. This requirement would be to strengthen the Cooperative Program committing each State to a formal course of action. Mr. Sherman, Connecticut State Department of Health, questioned if State officials would, in all cases, have the authority to commit themselves to such a course of action. It was agreed that Mr. Jensen would seek legal advice on this point and that unless advice to the contrary was received, the tentative draft of the manual would contain a statement requiring an official request for State participation.

2. PHS Program Reports to States:

Mr. Meredith, Virginia State Department of Health, suggested the proposal that the PHS submit quarterly reports to the States describing the progress in completion of the appraisal. He contended that in some situations more frequent reporting would be desirable, whereas in other instances, infrequent reports might be satisfactory. It was agreed, without objections, that the statement be revised to require that PHS review officer submit a trip report to the States.

3. Publication of State Ratings:

Mr. Jensen indicated that in his opinion, the publication of a list of specific State ratings would result in many misunderstandings which might be detrimental to the State agencies and the shellfish industry. Mr. Brown, Maryland State Department of Health, indicated that publication of identified State ratings would focus attention on those States whose programs were beginning to deteriorate. Mr. Jensen concurred but suggested the rating system was not yet sufficiently accurate to justify such conclusions. The problem was further discussed by Dr. Novak, Louisiana State University. Dr. Novak suggested that industry might attempt to use the slight differences in ratings to further the sale of their product. Other participants including Mr. Hobbs, Maryland State Department of Health, Dr. Buddington, District of Columbia, Department of Public Health, Mr. McIntyre, PHS Region IX, and Mr. Girard, Washington State Department of Health, suggested that the certification program was based upon the principle that a State program was either acceptable or not acceptable, and that relative State ratings should be of concern only to the State involved.

Mr. Sherman made a similar statement indicating that he would be interested in knowing his own State rating and its relative position to other State ratings, but that he was not interested in the specific ratings of other States. A similiar position was taken by Mr. Bellis, New Jersey State Department of Health. Mr. Bower, Washington shellfish industry, indicated opposition to a system which would result in the publication of comparative State ratings and urged that only statistical reviews be published. It was concluded that only a statistical summary of State ratings would be published by the PHS recognizing that experience may dictate some changes in this procedure.

Mr. Bower and Mr. Lehman, Delaware shellfish industry, pointed to the lack of controls exercised by many of the interior States, and stressed the need for adequate cooperation from these States to insure or prevent the sale of shellfish from non-certified sources.

4. Appeal and Review at a Low Rating:

The proposed procedure for appeal of low rating was adopted without discussion.

5. PHS Withdrawal of Individual Certified Shippers from List of Certified Shellfish Shippers:

Mr. Jensen explained that the developments during the past year demonstrated that situations could arise in which the Service might need to remove the name of a State certified shipper from the list of certified shippers. He pointed out that in some circumstances action could not be readily taken to withdraw endorsement of an entire State program, whereas the name of a single shipper could be removed from the list without economic damage to other State certified shippers, and with less severe political repercussion that might be expected from the withdrawal of endorsement of an entire State. He pointed out that the Service would take such action only after the State had been given an opportunity to first take such action.

State representatives, including Mr. Udell, Mr. Girard, Mr. Meredith, and Mr. Varn, Georgia shellfish industry, indicated that they were entirely opposed to such actions on the part of the PHS and urged that the Cooperative Program be administered on the basis of State program endorsement.

Mr. Lehman, Mr. Wallace, Director, Oyster Institute of North America, and Mr. Martin, PHS, Region II, pointed out that situations do develop in which it would be more practical for the PHS to suspend the certification of a single shipper rather than withdraw endorsement of an entire State program. Mr. Martin, in particular, pointed out that the withdrawal of endorsement might penalize innocent members of the shellfish industry for actions of a single shipper.

Mr. Fraizer, Ohio Department of Health, indicated that as a receiving State he could appreciate that a public-health emergency might arise in which the PHS might find it advantageous to remove a particular shipper from the list and suggested that without such power, there might be some question as to the integrity of the entire system.

Mr. Brown defended the principle of endorsement, and pointed out that if the PHS found it necessary to suspend the certification of a single shipper that this in itself was indicative of a fundamental weakness in the State program and the PHS should, in fact take the action against the State.

Mr. Bower pointed out that the PHS would undoubtedly have the power to either confiscate oysters or stop shipment in the event of a public-health emergency. Mr. Jensen, while agreeing with Mr. Bower, pointed to the fact that this placedthe PHS simultaneously in two opposed positions; i.e., confiscation of or interference with shipments of a plant with a valid shipping permit.

Mr. Gregory, Virginia Department of Health, noted that the State Health Commissioners generally have power to act in emergencies and that, in any event, if the PHS withdraws or suspends a certificate, that it must be recognized that it will be the Service's responsibility to reinstate the certificate.

Mr. Udell and Mr. Howell, Delaware State Board of Health, pointed to the need for the PHS to act in a public-health emergency in revoking such permits, with the emphasis being placed on the terminology of public-health emergency.

It was concluded that there was no agreement on this point and the problem should be discussed further at the regional shellfish meetings to develop a tenable position.

6. Responsibility of Rating Officer:

In the administration of the Cooperative Program, the PHS accepts the responsibility for the evaluation of the State program to determine the compliance of the existing standards; however, Mr. Jensen also pointed out that under such a Cooperative Program the States have a responsibility to assist the Public Health Service in obtaining the information. It was proposed, and accepted without objections, that the tenative manual require that the States present evidence to the PHS that the program is, in fact, satisfactory.

7. Application of Weighting System to the Evaluation of Sanitary Surveys of Growing Areas:

Mr. Jensen explained that the initial draft manual had been based on the application of the weighting technique to shellfish growing areas; i.e., the influence on the numerical rating is directly proportional to production volume. He explained that recent meetings with State personnel demonstrated that this approach contained some inherent weaknesses; and that from the epidemiological standpoint, small areas might well be as important as large areas. One recent outbreak of infectious hepatitis attributed to raw oysters being cited as an example of such a situation. He accordingly proposed that the review draft assign equal weights to each shellfish growing area and that the PHS try to develop an epidemiological weighting factor which could be applied to each growing area. This proposal was accepted.

8. Laboratory Evaluation:

Mr. Kelly, Chief, PHS Shellfish Sanitation Laboratory, and Mr. Jensen explained it was their opinion that adequate State shellfish sanitation programs depended upon adequate laboratory facilities. Mr. Kelly stated that a laboratory appraisal technique would be developed, and the report of laboratory reviews would be included in the PHS evaluation of State shellfish sanitation programs. This proposal was acceptable to the Workshop.

9. PHS Reports to State Control Agency:

Mr. Jensen explained that in many States more than one agency shared responsibility for the administration of shellfish sanitation activities. It was agreed that the States should determine if all PHS reports should be channeled through a single agency, or if parallel communication channels would be more desirable and should advise the PHS of the desired method of communications.

10. Sanitation Ratings for Intrastate Shippers:

Part I of the shellfish sanitation manual recommends that States have adequate sanitary controls for intrastate shippers. The program appraisal manual had recommended that a numerical sanitation rating be prepared for these shippers to determine if these State programs did have dual standards. On the basis of conversations with the States, it had been proposed that the inspection requirements be deleted, and that the review be limited to a study of the regulations applicable to intrastate shippers.

Mr. Wallace indicated that it was industry's opinion that dual sanitation standards was one of the weaknesses of the certification program and stated that this posed a great threat to both the program and industry.

Mr. Goggins, Maine Department of Sea and Shore Fisheries, indicated that Maine had dual standards but it was recognized that such dual standards were not necessarily in the best interests of the certification program.

Mr. Girard indicated that Washington did not have dual standards but he felt that it should be the right of the State to have such standards and that the PHS should not be involved.

Mr. Jensen then pointed out that dual standards in one State, which permitted bait digging of shellfish from polluted areas, had resulted in 13 cases of infectious hepatitis among people who used "bait shellfish" as food. In this case, a dual standard had hurt both the Cooperative Program, and the publicity was harmful to the legitimate shellfish industry. Mr. Udell asked if it would really be possible for the State to have dual standards, and suggested there would probably be interchanges of shellfish between the two classes of shippers. Mr. Mc K. Caldwell, North Carolina State Board of Health, indicated he favored a single standard, noting that intrastate certificates could be issued to those shippers who did not wish to participate in the intrastate program. Mr. Lauderdale, Texas State Department of Health, and Mr. Martin stressed the difficulties of evaluating a State program with dual standards and indicated that States must recognize that PHS officials would have to be much more critical in judging a State program which accepts lower standards for intrastate shippers. Mr. Jensen then referred to the detailed discussions of dual standards at the 1954 and 1958 Shellfish Sanitation Workshops, and reminded the Workshop that these previous discussions had lead to the agreement that a National program should not be used as a lever to force the adoption of uniform standards for local production, and to the adoption of the position. (Part I, Section A, Item 3, PHS Pub. No. 33.)

11. Credit for Effective Sanitary Survey:

Mr. Jensen explained that the draft of the review manual had contemplated a sliding scale which would give decreasing credits for sanitary surveys up to 10 years after the date of the original survey. He then explained that discussions with State agencies suggested that this approach was too liberal. In view of rapid changes which may occur in many of our coastal areas, it was proposed that this change would be changed to read as follows: 2 years-full credit; 3 years-85%; 4 years-10%; over 4 years-no credit.

Mr. Udell indicated that it was his opinion that survey data more than three years old has little value except for use in resurvey or reappraisal and urged that less credit be given. Mr. Brown and Mr. Mc K. Caldwell agreed with the proposition that old sanitary survey information needed to be brought up to date. However, no decision was reached and it was agreed that the above proposal would be followed, pending further discussion at the regional shellfish sanitation meetings.

The relationship between the initial sanitary survey and the reappraisal was raised by Mr. Brown, Mr. Martin, Mr. Caldwell, and Mr. Pierce, District of Columbia Department of Public Health. Mr. Jensen explained that the purpose of the reappraisal is to determine if the environmental changes in an area were such that the data obtained in the original survey would no longer reflect the sanitary quality of the area. If the "reappraisal" indicates that such changes have occurred, it would then be necessary to make a "resurvey", which might utilize data obtained in the initial "survey". The continued application of the reappraisal and resurvey techniques will keep the original survey current. This process is reflected in the Appraisal Manual.

Mr. Udell pointed out that from the point of State budgeting it is desirable to have definitive program requirements, and that there should be no question of the need for surveys, reappraisals, and resurveys at two year intervals.

Mr. Mc K. Caldwell stated that it was his opinion that there was a need for much better PHS guide for making sanitary surveys of shellfish growing areas. Mr. Jensen replied that an initial draft of such a guide had been prepared and should be available prior to the 1962 regional meetings.

12. Relationship of PHS Program Appraisals to Operations of State Judicial Systems:

Mr. Jensen emphasized it was not intended that the rating officer review the adequacy of the State judicial system, and that suitable changes would be made in the language of the manual. There was no objection to Mr. Jensen's suggestion on this point.

13. Preparation of Narrative Report:

Mr. Jensen indicated that it was intended that the rating officer prepare a complete narrative report for submission to the States near the end of each fiscal year. The proposal was acceptable to the Workshop.

14. Relative Importance of Boat Cleanliness:

Mr. Brinsfield, Maryland State Department of Health, suggested that the ratings 50, 20, and 10 for relative weights for boat cleanliness, truck cleanliness, and shell stock washing were not realistic. Mr. Jensen and Mr. Kelly indicated that in their opinion shell stock washing can be an important item, and that in areas such as Maryland, shell washing is practiced at the time of harvesting. Mr. Varn indicated that shell stock picked up by boat is almost impossible to wash. Mr. Pringle, South Carolina State Board of Health, indicated their experience indicates that at least 75 percent of the mud can be removed using shaker type washers. After considerable discussion, the Workshop agreed to the following inclusion of values in Form VII (Evaluation of Harvesting Practices) of the Appraisal Manual:

Construction and cleanliness of boats	50
Construction and cleanliness of trucks	25
Washing shell stock	5
Waste disposal from boats	20

In response to a suggestion, that the Workshop vote on the proposed values, Mr. Jensen indicated that it was his belief that it was not practical for the Workshop to vote on the adoption of specific items because of the lack of representation from some States, and the difficulties in defining the voting relationships between the Public Health Service, the several States, the Canadian Departments, and the shellfish industry. Mr. Wallace expressed the opinion that this vote was a departure from past procedures, which had accepted voting used in previous workshops. Mr. Jensen questioned if these earlier "votes" were meaningful, and raised the question of veto powers on the part of the PHS, the several States, the Canadian Government or the industry. The matter was not resolved.

15. The Evaluation of Extent to Which Cooperative Program Requirements Are Applied to Commercial Market Shellfish Harvesters and to Persons Handling Shellfish Prior to Intrastate Shellfish Shippers:

Mr. Udell pointed out that the proposed manual uses the system of porportional weighting in the evaluation of these two items (Part I, Section A, Items 2 b and c). He stated that it was his opinion that there was no justification for applying proportional credits to these program elements. After broad discussion it was agreed that this Section would be rewritten to eliminate proportional credits.

16. Interim Use of the Program Appraisal Manual:

Mr. Jensen again emphasized that the manual was still in a developmental stage, and suggested it be used on an interim basis for the next two years for the evaluation of State shellfish sanitation programs. He also suggested that experiences gained in this two year interval be reviewed at the 1963 Shellfish Sanitation Workshop. He stated that PHS would not take action against any State program on the basis of a rating below 70 percent unless there was evidence that something was seriously wrong with the State's program. The Workshop initially expressed no objection to this procedure. Mr. Udell then pointed out that he agreed with the suggestion that the tentative manual be used for State program appraisal during the next two years, but believed that arrangements should be made for an interim discussion in advance of the 1963 Workshop. Mr. Jensen agreed and indicated that the use of the manual should be reviewed by the several regional groups.

Mr. Brown then proposed that the Workshop approve the following statement for inclusion in the introduction to the manual:

> "The provisions of this manual were reviewed and tentatively accepted by the participants at the Shellfish Sanitation Workshop held in Washington, D. C., on November 28-30, 1961. It is intended and understood that the Public Health Service will be guided by the provisions of this manual in the appraisal of State shellfish sanitation programs and that experience gained by the use of this appraisal technique will be evaluated at the next Shellfish Sanitation Workshop. It is further understood that the Service will not act to terminate endorsement of any State because of a rating of less than 70 percent in any element of a State program unless there is concurrent evidence of a serious program weakness."

The Chairman, Dr. Novak, then requested the views of the group on the proposal. In the absence of any dissenting expressions, the manual was tentatively adopted for use until the next National Shellfish Sanitation Meeting.

Problems Related to the Sanitary Control of Shellfish Imports:

Mr. E. L. Ruppert, Public Health Service, presented a paper entitled "Public Health Service Position with Respect to the Sanitary Control of Shellfish Imports." This paper, which was to be presented by Mr. John Faulkner, Public Health Service, is included as Appendix C of these Proceedings.

Dr. Buddington inquired if the imported oysters were fresh, frozen, or processed. Mr. Ruppert replied that the statistics referred to fresh and frozen oysters but that substantial quantities of canned and smoked oysters are also imported.

The Chairman, Dr. Novak, then inquired if there was further discussion of this problem. In the absence of such discussions, the Workshop proceeded to the next agenda topic. (Note: A further discussion of this matter was held on Wednesday morning. For convenience this succeeding discussion is included at this point.)

Wednesday Morning - November 29, 1961

Mr. E. L. Ruppert- Chairman

Mr. E. L. Ruppert, Chairman opened the Wednesday morning meeting by indicating that discussions of the previous evening had led him to believe that some of the participants wished to reopen the discussion of the sanitary control of imports.

Mr. Gruble, Washington State shellfish industry, replied that he had requested that the subject of shellfish imports be reopened for discussion because he felt a failure to openly discuss the import problem would pose a serious threat to the continuation of the domestic cooperative shellfish sanitation program. He noted that his company (The Hilton Seafood Company of Seattle) is engaged in the processing and national distribution of seafood products and supports the Cooperative Program. He also stated that there was serious concern over the health incidents attributed to the shellfish and resulting adverse publicity. He stated that it was his opinion that the importation of shellfish from non-certified areas outside the United States or Canada was of great concern to the Cooperative Program. Mr. Gruble then stated that it was his impression that 15 to 19 interior States did not recognize the voluntary certification program and would allow the sale of shellfish from non-certified sources. It was his opinion that this dual program gave serious doubts as to the true value of such a voluntary program as a satisfactory public-health safeguard and that a lack of effective control over shellfish imports pointed to the need for corrective Federal legislation. As an immediate short term corrective measure, he recommended PHS initiate an effective publicity program to advise the public of the inherent dangers in the consumption of non-certified imported or fresh frozen shellfish. He cited the import statistics as evidence to the magnitude of the problem, and requested a full explanation of the steps the PHS intended to take to safeguard the public from playing "Russian roulette with shellfish".

Mr. Frazier stated that he had been charged with obtaining specific advice on what action the receiving State should take in respect to these imports. He stated that Ohio officials were relatively ignorant of the shellfish sanitation procedures and sought the advice of the personnel most acquainted with the problem.

Mr. Esveldt, Washington State shellfish industry, indicated that like Mr. Bower, he supported the Cooperative Program, but was appalled by the lack of consideration that had been given to this problem. He stated that it was his opinion that the Workshop group did not show good judgment if large amounts of time were spent in refinement of the Cooperative Program requirements within the United States while, simultaneously, ignoring the import problem or at best adopting a statement of policy. He stated that the shellfish industry was vitally concerned about some incidents attributable to imported shellfish causing a complete loss of public confidence in their product. He indicated that in his position he has contacts with wholesale buyers in about 40 major markets including such grocery chains as Kroger and A & P and that these corporations operate on the assumption that the official agencies would not permit the sale of a product if it was not fit for consumption. Mr. Esveldt indicated he thought it to be in the interests of the Public Health Service, the industry, and the future of the sanitary control program to ask the following specific questions:

1. Is the Public Health Service or the Food and Drug Administration prepared to state that non-certified imported frozen shellfish are safe for consumption by the public, that is, are they free from possible infectious microorganisms?

2. Does the Public Health Service or the Food and Drug Administration or the State Department have an interim control program to offer the industry as a temporary solution to this problem pending passing of appropriate legislation?

3. How can the Public Health Service reconcile the dual domestic and import standards from the public-health standpoint?

4. What specific measures are being taken to protect consumers in the 15 non-cooperating States?

5. Where are the present non-certified imported frozen shellfish being sold? Is the Public Health Service following these through to destination? Do they know where they are going; to what market; and to what use are they being put?

6. What would be the Public Health Service's position if a certified shipper sells frozen non-certified shellfish in the non-cooperating States?

Mr. Wallace added that the shellfish growers of the East Coast would also like to know the attitude of the producing States on this problem.

Mr. Spath, Louisiana State Board of Health, replied that Louisiana had outlawed imports of frozen oysters because they come from unapproved sources. The State does not feel they would be justified in certifying their own oyster beds and at the same time allow sale of oysters from uncertified areas in foreign nations. He indicated that they had not been successful in stopping the sale of canned oysters.

Mr. Pringle noted that only certified shippers may sell oysters in South Carolina. Mr. Dean, Georgia Department of Public Health, noted that they had a call from a Brunswick, Georgia, firm interested in import of oysters from Venezuela. They were advised they could not sell non-certified oysters in Georgia and were to contact PHS for more information. He also noted that last year there was a trucking firm in Atlanta that had reportedly made arrangements to import four million pounds of oysters a year from Mexico. It was his understanding that these plans were abandoned as a result of talks with the management.

Mr. Blair, Oregon State Board of Health, stated that Oregon did not authorize the importation of oysters from foreign points which are not included on the certification list.

Mr. Girard indicated that the State of Washington prohibited the marketing of oysters from non-certified sources. He explained that the Workshop should recognize that the real problem is in those interior States which may not have adequate laws for preventing the marketing of non-certified shellfish.

Mr. Gregory stated that their Department feels that a non-certified product is unsafe for sale in the State of Virginia, and it is believed that there is adequate law to prohibit its use.

Mr. Sarraf noted that Pennsylvania's regulations, specifically prohibit the importation of shellfish into the State of Pennsylvania unless the name of the shipper appears on the PHS certified list.

Mr. Mc K. Caldwell, Mr. Brinsfield and Mr. Bellis indicated that their regulations permitted only the sale of oysters from certified sources. Dr. Tennant, Canadian Department of National Health and Welfare, indicated that he was unable to state with assurance the position in Canada with respect to the importation of Japanese oysters. Mr. Lauderdale noted that Texas required the sale of certified oysters but that there were some problems with the importation of oysters from Mexico. Such oysters can enter and cross the State but, apparently, cannot be sold within the State.

Mr. Moorer, Mississippi State Board of Health, indicated that only the product of shippers on the PHS list of certified dealers may be sold. Mr. Frazier explained that under Ohio's regulations shellfish which are sold in commercial operation, such as in restaurants must be from the PHS list of approved shippers. On the other hand, he noted that shellfish which were sold through grocery stores, and therefore under the inspection of the State

Department of Agriculture, need not be from the approved list. He further noted that many local health departments would require that all shellfish sold in their jurisdiction be obtained from the certified list.

Dr. Slocum, Chief of Microbiology Food and Drug Administration, explained the position of the Food and Drug Administration. The Food, Drug and Cosmetic Act requires that products offered for entry into the United States must comply with the law. There is no provision for foreign inspections; thus, the Administration must evaluate the legality of the products as they are offered at customs for entry into this country. He noted that the Food and Drug Administration had examined practically every lot of imported frozen shellfish utilizing the bacteriological standards of the Cooperative Program. A great many of these shipments had been detained. He also noted that their results have shown a drastic improvement in bacteriological quality.

Mr. Wallace expressed his position with respect to this problem. He noted that it was their position that objective tests by the Food and Drug Administration at the port of entry were not a substitute for the kinds of controls contemplated in the Cooperative Program. The entire concept of the shellfish sanitation program in the United States and Canada is a series of controls from the time of growing, harvesting and handling, and that since 1925 this program has been built up at a tremendous cost to the various States. He then charged that the Workshop could not simultaneously take the position that stringent sanitary controls were necessary for shellfish produced within the United States, while at the same time, taking no action to require similar controls for shellfish produced outside the United States. He indicated that the industry has a strong representation at the Workshop that was indicative of their interests in the Cooperative Program, and urged very strongly that the same basic concepts be applied to shellfish that might be produced anywhere in the world for shipment into the United States. He added that any shellfish to be sold to the American public should afford the same degree of protection as is given the domestic product.

Dr. Buddington inquired of Mr. Wallace if he would accept the cooking of the frozen imported oysters as a satisfactory practice. Mr. Wallace replied that it would not be acceptable. He then pointed out that it has been the practice in the United States to prevent the canning of polluted shellfish. He also cited an incident in Virginia in which several thousand gallons of oysters had been harvested from areas which was found to be polluted. When the industry requested permission to can these polluted oysters, the State refused and the oysters were destroyed.

Dr. Buddington stated that it was his impression the Federal and State inspections in foreign waters would not soon be forthcoming and questioned what interim steps could be taken. Mr. Wallace replied that he felt that foreign inspection would be prefectly practical and that he hoped that appropriate Federal legislation would be adopted to facilitate this type of program. He noted that the President's international policy is to foster trade with foreign countries, and that it was his opinion that foreign nations would assert continuous pressure until arrangements could be made to facilitate such marketing. He stressed the need for urgency and cited the infectious hepatitis incidents of 1961 as evidence of the need for satisfactory controls.

Mr. Bower explained that the shellfish industry as a whole had recognized their obligations to the consumer public, and emphasized the danger inherent in the growing and marketing of shellfish without sanitary supervision. He noted that thousands of pounds of imported shellfish are now entering the United States and that there is no sanitary control over the growing and packing operations. He stated that the West Coast industry felt that the continued sale of uncertified shellfish posed a health hazard to the consumers, and an economic hazard to the shellfish industry because of possible loss of confidence which might result from a disease outbreak. He urged that as an interim measure, consumers be made aware of the dangers inherent in the use of uncertified shellfish. He noted that, if necessary, this can be done by the shellfish industry without the cooperation of the States and Federal agencies. Mr. Brown, Maryland State Department of Health, suggested that the Workshop develop a position statement with respect to the sanitary control of shellfish imports. He suggested that such a statement might include the following points:

1. Reaffirm the need for sanitary controls of shellfish which will be sold as fresh or frozen products.

2. Recognize that shellfish imported into this country are not subject to such controls.

3. Recognize there is no mechanism for establishing such controls over shellfish imports and urge that the United State Government take steps to extend the controls of interstate certification to shellfish to be imported into this country.

4. Recommend that consuming States adhere to the principles of the present interstate certification program to protect their populations.

Mr. Kuwahara, shellfish industry of Japan, pointed out that he and his colleagues were very pleased to attend the National Shellfish Sanitation Meeting in the United States. He explained that Japan is now producing five million gallons of oysters a year and that they recognize the value of improving sanitation standards in the shellfish industry. He noted that in 1954, Japan requested the United States Government to send technical consultants to Japan to give information on every important producing area. At the present time, it is very difficult or impossible to apply United States control measures generally in Japan; however, Hiroshima Prefecture has adopted rules and regulations governing the control of fresh oysters for domestic consumption and last year adopted very strict rules and regulations for shellfish for export. He explained that the Japanese shellfish industry would like to be in a position to deal with this country on an equal level.

The Chairman, Mr. Ruppert, summarized the discussions, suggesting that Mr. Brown, Mr. Wallace, and Mr. Esveldt get together later to draft a Workshop position statement on the sanitary control of shellfish imports. He then asked that Mr. Jensen and Dr. Slocum answer the specific questions posed earlier by Mr. Esveldt (page 8). He noted that in reply to Mr. Esveldt's last question relating to handling of non-certified shellfish that a certified dealer could not handle shellfish which were not from non-certified sources. Dr. Slocum explained that the Food and Drug Administration was a party to the 1925 agreement establishing the Cooperative Program. In the absence of similiar measures in foreign countries, the Administration must rely on bacteriological analysis. While the limitation of such examinations are recognized, they do give the Administration a substantial amount of information about the sanitary quality of the product. He pointed out that none of the products which had been permitted entry, as far as he knew, had been involved in disease outbreaks. He acknowledged that many of the products were undoubtedly going into processed seafood products so that the hazard of diseases, such as infectious hepatitis, would be minimized.

In answer to Mr. Esveldt, Mr. Jensen explained that the Public Health Service is committed to the philosophy of control at the source and that it cannot recommend the sale of shellfish from non-certified sources. He pointed out that this position had been stated repeatedly, and explained that it was not possible for the Public Health Service to prohibit the sale of shellfish from non-certified sources. The Service can, however, inform interior States of the hazards of shellfish from non-certified sources. Mr. Esveldt stated that this did not answer his question, and that in his opinion bacteriological examination would not substitute for control of source by the Cooperative Program. Mr. Jensen agreed that this was so, and pointed out that naturally occurring toxins and chemicals might also be present which would not be disclosed in bacteriological examinations of the products. He again explained that PHS could not recommend the sale of shellfish from non-certified areas, but that under the framework of the voluntary program it was up to the States, who have both rights and responsibilities, to take such actions. Mr. Ruppert added that the Service cannot reconcile the use of dual domestic-import standards. Mr. Esveldt then suggested that PHS present this information officially to all health agencies in the non-participating States.

Mr. Jensen indicated that the Service had made an increasing effort to interest the interior States in better use of the PHS list of State-certified shippers. He noted he had visited food control officials in Kansas, Missouri, Minnesota, and Iowa during the past year and had found some of the inspectors in the State departments of agriculture were very poorly acquainted with the Program. He also noted that the Public Health Service has food sanitation staff members in Kansas City, Chicago, and Denver offices who could help in this respect. Mr. Van Derwerker, Region V, PHS, noted that some of the large city health departments sample practically every lot of shellfish. He indicated that at least in one instance a dealer (Chesapeake Bay) was denied further entry to the Chicago market on the basis of unsatisfactory bacteriological results. With respect to the proposed action, Mr. Van Derwerker agreed that a resolution as proposed by Mr. Brown would, if presented to the Association of State and Territorial Health Officers, help promote a general recognition of this problem, and expand the use of the certified list in the interior States.

Mr. Ruppert advised that the matter had been discussed previously with the Association of State and Territorial Health Officers and the Conference of State Sanitary Engineers. Both organizations have gone on record as recommending more adequate control on imports, and that only certified shellfish be sold. Mr. Ruppert suggested that Mr. Frazier also participate in the development of the position statement.

Mr. Esveldt noted that a statement had been made that thousands of pounds of uncertified clams were being brought into California and that it was assumed that these were going to be utilized in a processed product. He stated that it was his opinion that FHS should make a real effort to follow these non-certified shellfish to the ultimate market.

Mr. Wallace added that the imports statistics showed that shellfish were brought into areas other than California, for example, New York. Mr. Udell explained that New York State did not permit the sale or distribution of non-certified shellfish; however, the shellfish may enter through the Port of New York, but must remain in bond until shipped to another State.

Mr. McIntyre explained that the California State Department of Health (not represented at the Workshop) does not allow the sale of non-certified shellfish for raw consumption. However, information does not always get through to the State Health Department when a non-certified product passes through the port. Dr. Orcutt, California Department of Fish and Game, indicated that they knew that imports were coming in but that he had no knowledge of what the State public health organization was doing in this regard. He indicated, however, that the local shellfish industry was aware of these imports and that they were in competition with shellfish which were produced under lesser sanitary controls and therefore lower costs. Mr. Kuwahara explained that the total quantity of fresh shellfish which might be exported from Japan would not be large, probably not more than 200,000 gallons per year.

Thursday - November 30, 1961

A Committee, consisting of Messrs. Wallace, Frazier, and Brown, drafted and presented the following relative to the importation of fresh and frozen shellfish:

The Fourth National Shellfish Sanitation Workshop has adopted the following statement of position with regard to the importation of fresh and frozen shellfish:

1. The full range of control of shellfish--through growing, harvesting, processing and marketing--developed over the past 35 years in the Cooperative Interstate Shellfish Certification Program is necessary to protect the health of the shellfish consuming public;
2. Imports of fresh and frozen shellfish are now being made, with such shellfish not being subject to the controls provided in the said Shellfish Certification Program; consequently, sole reliance upon physical, bacteriological and chemical examination of the shellfish at the point of entry, or market, does not insure absence of hazards to health of the consumer;
3. Necessary legal, fiscal, and administrative bases required to enable extension of the said Shellfish Certification Program to foreign sources of shellfish do not presently exist;

4. It is urgently necessary that the U. S. Government take action to make possible those measures required to extend control of the Shellfish Certification Program to foreign sources of shellfish;

5. It will be further necessary in order to insure protection of the health of the consuming public from hazards presented by shellfish from uncertified sources, that all States not presently participating in the said Shellfish Certification Program be alerted by the Public Health Service to the hazards that exist and be encouraged to accept and be governed by its provision.

The Chairman, Mr. Glude, then requested comments from the floor. Dr. Novak, suggested that the word "immediate" be inserted (between the words "take action") in Item 4, line 1. Mr. Brown replied that it was the opinion of the Drafting Committee that the word "urgently" as used in the first portion of the sentence conveys his feeling. Mr. Hasegawa, Agriculture Attache, Embassy of Japan, proposed that the order of Items 4 and 5 be reversed and that the word "further" in the first line of Item 5 be deleted. Mr. Gruble raised the question as to the authority of the Japanese to participate in the Proceedings. Mr. Jensen replied that Mr. Hasegawa represented the Embassy of Japan, but that the Japanese were invited to this Conference only as observers and that his suggested change was only a personal recommendation. Mr. Hasegawa confirmed Mr. Jensen's statement. In the absence of further discussion, the Chairman ruled that the statement of position would be adopted by the Workshop as amended.

Mr. Hasegawa, after informal conferences with Mr. Jensen, proposed that the preamble to the position statement be rewritten as follows:

> "The Fourth National Shellfish Sanitation Workshop adopted the following statement of position with regard to the sanitary quality of fresh and frozen shellfish exported to the United States."

It was Mr. Hasegawa's contention that this would explain the Conference was concerned only with the sanitary quality of shellfish, and would recognize that the use of the word "imported" might cause some misunderstanding at the international level. After extensive discussion it was agreed that this change was not acceptable to the Workshop and the position statement stood as originally adopted.

Report on Hot Dip (Shock) Method of Opening Oysters:

Mr. Somers B. Pringle, Sanitation Consultant, Shellfish Section, South Carolina State Board of Health, presented a paper entitled "The South Carolina Report on the 'Hot Dip' or 'Shock' Method of Opening Oysters". Mr. Pringle's paper is included as Appendix D of these Proceedings.

Mr. Dean inquired if the oysters had a milky appearance after shocking. Mr. Pringle replied that there is no evident change if the time-temperature relationship is properly controlled.

Mr. Ruppert noted that the Cooperative Program standards did not have specific water temperature, time and cooling requirements applicable to this method of opening oysters. He indicated that if "Hot Dip" was to be an official method, sanitary standards would eventually need to be incorporated in the manual of recommended practices. He also raised the question as to whether the product could be considered as a fresh oyster. If they are not so considered, they would then not come under the provisions of the voluntary program which is limited to fresh and frozen shellfish. Mr. Pringle replied that there was no apparent change in the physical characteristics of the oysters and noted that the consumer public in South Carolina accepts the product as a fresh oyster. In the absence of a change in appearance or flavor and taste, it was his opinion that South Carolina "Hot Dip" shucked oysters should not be considered as a processed oyster. With respect to the time-temperature relations, South Carolina studies indicated that 150°F. was the minimum temperature and that two minutes was the maximum exposure time, with prompt cooling being essential. Mr. Pringle did not specifically request this formula be included in the Manual, but he indicated this procedure should either be incorporated in Part II of the Manual or should be readily accepted by receiving States as a normal certification if certified by the shellfish State of origin as meeting all the requirements of the Manual.

Mr. Ruppert noted that some time-temperature limitations would be desirable; as higher temperatures or longer times would undoubtedly produce changes in the organoleptic character of the shucked shellfish.

Mr. Mc K. Caldwell indicated that he did not object to Mr. Pringle's proposal, other tha to urge that these standards not be incorporated in the Manual until further studies could be made. He indicated that limited studies in North Carolina suggested the South Carolina temperature-time relationships are not satisfactory from the standpoint of the shucker, who preferred temperature of 150°F to 160°F with times of exposure of 6 to 10 minutes. He also noted that oysters which were chilled to 40°F opened readily with $1\frac{1}{2}$ or 2 minutes holding time. Mr. Bower noted that different time-temperature relationships would probably be required for different species in different localities.

Mr. Hobbs noted that the ratio of water to oysters would also be a consideration. Mr. Pringle replied that in South Carolina the process had been used only by small shuckers (25 gallons per day, 90 percent sold intrastate) and that no attempt was being made to sell the process for use in the larger plants. He also noted that oysters should be opened as soon as possible after "shocking", preferably within 30 minutes. He noted the process had been used in small quantities half bushels, but had found it did not work satisfactorily with three bushel containers. He added that the State of South Carolina had adopted sanitary regulations for these plants.

Mr. Wallace explained that the industry was interested in any process which would improve their operations and suggested that there should be no basis for denying use of the process in the absence of any evidence that the product suffered any deterioration from a sanitary standpoint.

Dr. Tennant agreed with the earlier comment that time and temperatures have to be worked out for each location and product. He reported that in his previous work with soft shell clams, a water temperature of 200°F and an immersion time of 3 to 30 seconds was found to be adequate. He also noted that mud would accumulate in the vat and that some standards for water changes might be necessary.

Mr. Mc K. Caldwell inquired if the hot dip method was used in Georgia or Florida. Mr. Dean replied that the method was not used in Georgia. Mr. Pringle noted that he had received two requests from the State of Florida for information on the method.

Mr. Mc K. Caldwell noted that North Carolina Food and Drug officials believed that this product should carry some designation other than fresh shucked oysters. This position might be modified depending on the positon taken by the Public Health Service with respect to the use of the product.

Dr. Slocum explained that the Administration had not taken a formal position on this, but if it appeared that the oyster meats were actually cooked, then a different designation would be necessary. He noted that Mr. Pringle's statement indicated that the characteristics of the fresh oyster were retained.

Mr. Jensen suggested that it would be appropriate for the Workshop to go on record as accepting the continued use of the shocking process as now practiced in South Carolina with the caution that the process not be adopted in other States without prior studies to define the time-temperature relationships necessary with the particular species of shellfish being handled and that no change be made in the shellfish sanitation manual at this time.

Mr. Wallace and Mr. Varn agreed with this proposal; however, Mr. Sherman stated that he believed this was a problem between the PHS and South Carolina and that he personally did-not feel that most of the States were sufficiently well acquainted with the process to take a position with respect to its use.

Mr. Ruppert replied that it was the opinion of the PHS that other States should know of the process, and should recognize that it was currently being used in the State of South Carolina with the concurrence of the PHS. He summarized the Workshop position as follows:

1. That no standards be adopted at this time in the Cooperative Program.

2. That the hot dip process be considered as interim process.

3. The limitations of the process makes its inclusion in the Manual unnecessary.

In the absence of objection, Mr. Ruppert's summary was considered to represent the opinion of the group.

Need for Sanitary Controls of the Scallop Industry:

Mr. Ross Homan, Chief Inspector, Canada Department of Fisheries, presented a paper entitled "Sanitary and Quality Control of the Canadian Scallop Industry". Mr. Homan's paper included as Appendix E of these Proceedings.

Mr. Frederick Wilbour, Department of Natural Resources, Division of Marine Fisheries, Boston, Massachusetts, then presented a paper entitled "Sanitary Control of the Massachusetts Scallop Industry". Mr. Wilbour's paper is included as Appendix F of these Proceedings.

Mr. McFarren, Chemist, PHS Sanitary Engineering Center, Cincinnati, inquired as to the meaning of shucking, and indicated that he had heard of a proposal to bread whole scallops. Mr. Wilbour replied that only the abductor muscle is used as a food product; however, Mr. Homans added that in England there is a market for the entire scallop, including the viscera, and that the product is not acceptable without the viscera.

Mr. McFarren pointed out that the visceral mass of scallops may contain substantial quantities of paralytic shellfish poison; however, he indicated that he did not know if it was true of all scallops or only those from certain areas.

Dr. Tennant, Canadian Department of National Health and Welfare, explained that scallops in the area referred to by Mr. Homans had been found to be highly toxic, but that the meats (the abductor muscle) is not toxic.

Mr. Shaw, Massachusetts Department of Natural Resources, indicated that three or four years ago a former chef on the Andrea Doria had been interested in preparation of a breaded product which would include the viscera of sea scallops. He was apparently unable to obtain backing for this project and it was abandoned.

Mr. Jensen, Public Health Service, explained that when the shellfish certification program was started scallops were included, but that they were no longer included. He indicated that over a period of years several States had noted the unsanitary conditions in the scallop industry and suggested that the Cooperative Program again be expanded to include scallops. He said the Service had been reluctant to act in this area, but that if there was a substantial State interest, the Service could consider the development of such a program.

Mr. Glude, Fish and Wildlife Service, indicated that he did not know of an official Fish and Wildlife Service position with respect to the sanitation of the scallop industry, but that it was his opinion, particularly in view of the occasional association of paralytic shellfish poison with scallops, that the certification program should consider scallops as "shellfish".

Mr. Homans and Dr. Tennant both pointed out that this poison had been found only in the visceral mass and that in the United States and Canada, at least, this is not used as a food product.

There were no further comments from the Workshop group as to the desirability of including scallops in the shellfish certification program.

Proposed Changes in Parts I and II, Shellfish Sanitation Manual:

Mr. Lee Roy Lunsford, Public Health Service, introduced the subject, pointing out that it has been the practice to make continuing adjustments in the Cooperative Program shellfish sanitation manual to reflect changing conditions and new technical information. He indicated the changes which were proposed would be published as supplement or new pages for the existing Parts I and II of the shellfish manual. The proposed changes were received from many sources including industry, State control agencies, and the regional seminars, (Regions I and II Shellfish Sanitation Coordinating Conference, Chesapeake Bay Seafood Seminar, Gulf Coast Seafood Seminar, Conference of the West Coast Shellfish Sanitation Authorities). Additional changes have been suggested by the Public Health Service on the basis of experience with the administration of the certification program since the last revisions of Parts I and II in 1958 and 1956, respectively.

Discussion of Suggested Changes

PART - I

1.* Suggested Change, Section A, Item 1, Page 3: That this item be rewritten so as to prohibit the harvesting of shellfish for direct marketing from any areas not approved specifically for this purpose.

Discussion: The requirement that shellfish not to be harvested for direct marketing from any area until a sanitary survey is made, is one of the oldest standards in the Cooperative Program. A report entitled, "Report of Committee on Sanitary Control of the Shellfish Industry in the United States", September 28, 1925, outlined recommended measures for the sanitary control of the shellfish industry. The first recommendation states:

> "That only those shellfish should be marketed which have come from beds which, on careful examination, are found to be free from any justifiable suspicion of dangerous contamination with disease producing microorganisms and free from such other contamination as might be in any way deleterious or offensive . . ."

It is apparent that emphasis is to be placed on the study of areas which are open rather than on a study of areas which should be closed. The hazards involved when approval is not used, have been clearly illustrated in widely separated incidents in which marketable quantities of shellfish have developed in previously barren areas. As it is now generally known, the development of a new oyster reef off the mouth of the Pascagoula River had disastrous consequences. Even on the West Coast, where Pacific oysters are supposed to grow only where planted, it has been necessary to officially close Vancouver Harbor because of an unexpected "set".

It is accordingly recommended that the Workshop reaffirm the previously expressed policy that shellfish be harvested only from Approved areas and that suitable changes be made in the manual to emphasize this position.

Recommendations: That the following seven changes be made.

(1) Section A-1 "State Laws and Regulations", page 3: Change sentence to read as follows:

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* Numbers refer to Suggested Changes, Discussion, and Recommendations listed in Mr. Lunsford's paper, copies of which were distributed at the Workshop.

"This legal authority shall enable one or more department or agencies of the State to classify all coastal waters for shellfish harvesting on the basis of sanitary quality."

(2) Section A-1, "Satisfactory Compliance" Item a, Page 3: Revise the first sentence as follows:

"Classify all coastal waters as to their suitability for shellfish harvesting on the basis of sanitary quality as defined in Section C of this manual."

(3) Section A-1, "Public-Health Explanation", Page 3: Insert the following paragraph at the end of the present public-health explanation:

"Experience has demonstrated that all coastal waters of the State must be classified as to their sanitary suitability for shellfish harvesting. Harvesting should be permitted only from those areas which have been found by sanitary survey to meet the sanitary criteria of this manual. Harvesting should accordingly be specifically prohibited from areas which do not meet the criteria, or which have not been surveyed."

(4) Section C-2, "Classification of Growing Area", Page 11: Change sentence one to read:

"All coastal waters shall be classified as to their public-health suitability for the harvesting of marketing shellfish."

In line seven insert the word "upward" before the word "revision". Similar changes should also be made in Items (a) and (c) of this item and in lines five and eight of the public-health explanation.

(5) Section C-2, "Classification of Growing Areas", Page 11: A new item "d" should be added to the section as follows:

(d. "All coastal areas which have not been subjected to sanitary surveys shall be automatically classified as Prohibited."

(6) Section C-6, "Prohibited Areas", Page 18: The following sentence be inserted as a new second sentence in this paragraph.

"Coastal areas which have not been subjected to sanitary survey shall be automatically classified as Prohibited."

(7) Section C-6, "Satisfactory Compliance", Page 18: A new item "c" should be added to the section as follows:

(c. "Coastal areas in which sanitary surveys have not been made shall be automatically classified as Prohibited."

It is recognized that adoption of this concept will pose some problems for some States with respect to identification of closed areas. In view of the recent experiences of the shellfish program, and the continued population growth in the coastal areas, it is believed that the Cooperative Program has no choice but to adopt this more conservative approach to the classification of shellfish growing areas. It is recommended that legal counsel be consulted in each of the States to determine how non-approved areas may be best identified.

In this connection, the States should also give some consideration to the need for protecting picnickers or sport fishermen from inadvertently harvesting shellfish from areas which are of unsatisfactory sanitary quality.

Mr. Pringle questioned if these changes would mean that the States would have to make sanitary surveys of all coastal areas even though they had not been productive of shellfish for many years. Mr. Gregory, Virginia Department of Health, pointed out that the proposal was the opposite of current State practice, i.e., the State examines areas which they feel are in question, and that areas found to be polluted are so classified. He pointed out that this procedural change would require legislative changes and would cause administrative problems in classifying and delineating all areas.

Mr. Wilbour, Massachusetts Department of Natural Resources, pointed out that in Massachusetts hundreds of thousands bushels of hard shell clams have been taken out of an area in Nantucket Sound which is beyond the territorial limits. He questioned if the State agencies had a right to go outside of the territorial limits to examine such an area.

Mr. Girard, Washington State Department of Health, pointed out that Washington's law requires the State agency to approve specific areas and permits the sale of shellfish only from such approved areas. He then went on to express his opinion that the proposed changes would require that the State survey almost three thousand miles of shore line, many of which could never be used for the production of shellfish. Mr. Udell, New York State Department of Conservation, indicated that the New York State law, like that of Washington, required the approval of specific areas with all other areas being automatically classified as restricted or condemned. He also concluded that the purpose of the change was in some way different from that now being used by his Department. Mr. Jensen then questioned Mr. Udell as to the operation of the New York State law, and it was developed that the State was currently classifying all coastal waters.

Mr. Brinsfield, Maryland Department of Health, indicated that the problem might be solved by inserting the words "shellfish bearing areas" suggesting that it would not be necessary to survey areas which did not produce shellfish. Mr. Silva, Region IV, replied that this was not a feasible approach and that it ignored the experience in Mississippi in which a supposedly non-producing area had produced commercial quantities of shellfish and had resulted in an outbreak of infectious hepatitis. He stressed the importance of having all State waters classified and that State program evaluations stress the approval of harvesting from only specific areas.

Mr. Brinsfield again reiterated his opinion that the State could not classify an area as restricted unless they had previously made a sanitary survey which would show the classification was justified. He again stated that it would be impractical for the State to study all of its growing areas.

Mr. Goggins, Maine Department of Sea and Shore Fisheries, explained that in the Western part of the State it was more convenient to describe the four or five open areas than the 15 to 20 closed areas, but that this system had not yet been applied in the Eastern portion.

Mr. Jensen then pointed out that the Cooperative Program should be so managed that there would be assurance that shellfish would not be harvested from areas which had not had satisfactory sanitary survey. He explained his opinion that this objective could not be achieved by a State program which emphasized the examinations of areas for closures, rather than examination for approval, and urged that the positive aspects of area study be stressed.

Mr. Brinsfield again stated that there were areas in the Chesapeake Bay which were not classified, but which although once productive of oysters were no longer productive. Mr. Jensen inquired if it was Mr. Brinsfield's opinion that there should be potential shellfish production areas in Maryland which were unclassified, that is were neither open or closed. Mr. Brinsfield replied that it was not his opinion that this would be desirable. He amplified his statement by indicating that it was easier to define the closed areas because there was only a few of them but he again agreed that there were potential oyster producing areas which were not classified as either closed or open.

Mr. Howell, Delaware State Board of Health, stated that it was his understanding that the proposed change simply called for classification of all areas instead of ignoring areas because of the lack of information as to the quantity of commercial shellfish potential as shellfish growing areas.

Mr. Sower pointed out that the State of Washington had many miles of coast line and that it would be a long time before the State could make all of the required surveys. He indicated that razor clam diggers were now, perhaps including as many as 100,000 tourists, taking clams from areas which had not been surveyed.

Mr. Bellis, New Jersey State Department of Health, indicated that the State had surveyed their entire coast line and that there were no unclassified areas.

Mr. Mc K. Caldwell, North Carolina Department of Health, indicated that his State uses a system similar to Maryland in which all areas of the State are approved unless otherwise designated. He also indicated that the Department of Conservation can close areas for conservation purposes. He stated it was his opinion that the proposal would pose administrative problems for the State control agencies because of the need for marking every area with identification signs.

Mr. Brinsfield inquired if it was the position of the Service that the State should have some knowledge of all coastal waters. Mr. Jensen replied that it was the position of the PHS that all coastal areas must be classified, i.e. any areas not surveyed should be classified "prohibited" to assure against harvesting.

Dr. Novak, Louisiana State University, explained the State had a extremely long coast line and that it would be impractical for them to undertake to complete a survey of this coastal area. Mr. Jensen inquired if it was Dr. Novak's suggestion that the State Board of Health approve the harvesting of oysters from areas without knowledge of prevailing sanitary conditions. Dr. Novak replied that he would not suggest such harvesting and indicated that it was his understanding that the Public Health Service proposal required that the entire coast be surveyed. Mr. Jensen replied that it was intended to make it clear that shellfish should be taken only from areas that had been surveyed and to prevent harvesting from all other areas not designated by the State as approved. He noted that posting would be necessary in those areas in which there are marketable quantity of shellfish to prevent harvesting. He emphasized that there is no intention to require a massive survey of the entire coastal area of Louisiana or any other State. Dr. Novak indicated that he could not object to such a proposal.

Mr. Goggins indicated that they had recently closed a potential producing area because of pollution although there had been no clams taken commercially from the area for 50 years.

Mr. Lunsford again pointed out that the requirements do not present a change in the shellfish sanitation manual, but only emphasizes the existing requirements of Part II. Mr. Jensen summarized the discussion pointing out that in a negative sense the proposed rewording had been accepted unless someone registered objection. In the absence of objections, the proposed changes with regard to harvesting from approved areas were considered as accepted. There was no further discussion on this item.

2. Suggested Change, Section A-1, "Satisfactory Compliance", Item g, Page 3: It is suggested that the sentence now enclosed in parenthesis be removed from parenthesis and restated as follows:

> "Hearings or other administrative procedures required in connection with such emergency actions should not require more than one day to complete."

Discussion: Experience has indicated that this is an important aspect of a State control program, and that it should not be thought of as a parenthetical after thought.

Recommendation: It is recommended that the suggested change be made.

No objection was expressed to the adoption of this proposal change.

3. Suggested Change, Section A, Page 4: It is suggested that a new item be added requiring that where two or more agencies are involved in a State shellfish sanitary control program, that a written agreement be developed outlining the responsibilities of each agency.

Discussion: In nearly every producing State responsibilities for sanitary control of the shellfish industry is shared by two or more State agencies. Each agency must have a clear understanding of the relationship between its responsibility and that of the other participating agencies if the overall State program is to function effectively. Again, experience has shown that poor cooperation between State agencies may result in significant public-health hazards. It is believed that this can be minimized through the development of interdepartmental memorandum of understanding. It is pointed out that such agreement has been developed between the Public Health Service-Food and Drug Administration, Public Health Service-Fish and Wildlife Service.

Recommendation: That the following be entered in Section A, Item 2:

> "An interagency memorandum of understanding shall be developed when two or more agencies are involved in the sanitary control of the shellfish industry in a State."

Satisfactory Compliance, Section A, Page 4: This item will be satisfied when interdepartmental memoranda of understanding have been developed which will define the responsibilities of each State agency in maintaining adequate sanitary control of the shellfish industry.

Mr. Shaw, Massachusetts Department of Natural Resources, pointed out that the responsibilities of the various departments were defined by legislation, and that there is no stipulation for agreements with Department of Health or other Departments. Mr. Pringle, South Carolina State Board of Health, indicated that recent experience in South Carolina as to the responsibility for patrolling made the developments of such agreements highly desirable.

Mr. Howell, Delaware State Board of Health, pointed out that Delaware had developed an interagency memorandum approved by the heads of the two State agencies responsible for shellfish sanitation program. The memorandum of agreement has clarified the responsibility of each agency and has contributed to the program effectiveness.

Mr. Moorer, Mississippi State Board of Health, explained the recent experience in Mississippi. He indicated that it had been their feeling there had been relatively close operating relationships between the conservation and health departments; however, he noted that personnel changes, particularly in the conservation department, made it difficult to maintain adequate communication lines. As an example, he noted that of the 15 fishery inspectors on the Marine Conservation Commission, only three of the senior inspectors had been carried over from the previous Commission. It was his opinion that a mutual agreement pinpointing the responsibilities of each department is highly desirable and indicated that the State had recently developed such an agreement.

Mr. Glude, Fish and Wildlife Service, stated that it was his opinion that the purpose of such an agreement was to clarify the responsibilities of the agencies involved and suggested that the statement be reworded as follows:

> "When two or more agencies are involved in the sanitary control of the shellfish industry, a clear statement of responsibility of each agency shall be developed."

Mr. Macomber, Region II, PHS, indicated that it had been his experience that agreements of this nature were needed in most States, and that although such an agreement might not have legal standing, it would help to define the areas of responsibility of the agencies and would assist the Public Health Service in the evaluation of State shellfish sanitation programs.

Mr. Martin, Region II, PHS, pointed out that this is not a hypothetical situation and that in one State three State agencies share responsibilities. He stated that it had been the regional experience that in almost all cases where more than one agency is involved that there is not a clear understanding on the part of either agency as to the areas of responsibility. He stressed that PHS was not seeking the development of a legal document, but was strongly urging development of a written statement which would define the areas of responsibility. Such a document would not be legally binding and it would not give away an agency's responsibility.

Mr. Fayard, Mississippi Department of Conservation, indicated that they have recently worked very closely with the Mississippi Department of Health and that satisfactory progress has been defining these areas of responsibility.

Mr. Lunsford pointed out that a memorandum of understanding had been developed between the Fish and Wildlife Service and the Public Health Service and between the Public Health Service and the Food and Drug Administration to outline the different areas of activities.

In the absence of further discussion the proposed change, as amended by Mr. Glude, was accepted.

4. Suggested Change, Section A, Item 2 h, Page 6: That the necessary inspection equipment be listed in this item.

Recommendation: That item 2 h be changed to read as follows:

> "State shellfish plant inspectors are provided with the following inspection equipment: Standardized inspection forms, thermometer, chlorine test kit, and light meter."

The proposal was adopted without comment.

5. Suggested Change, Section C-3, Page 13: It is recommended that the Pascagoula infectious hepatitis incident be mentioned in connection with the public-health explanation.

Mr. Moorer objected to the direct reference to the Pascagoula River incident, and urged this be deleted. Mr. Moorer's proposal was adopted without further discussion.

Mr. Ruppert and Mr. Macomber also suggested that the reference to coliform levels might be premature and urged its deletion. The Workshop was in agreement with this proposal.

6. Suggested Change, Section C, Item 3 c, Page 13: That this item be rewritten to indicate that the 230 MPN refers to a 5-tube dilution, and that an MPN of 330 can be accepted where a 3-tube decimal dilution is used.

Discussion: The statement that not more than 10 percent of the samples ordinarily exceed an MPN of 230 per 100 ml., is applicable only to the 5-tube decimal dilution test. Since many States use the 3-tube decimal dilution test, we agree that equivalent statistical limits should be set for this test.

Recommendation: That the first sentence in Item 3 c, be changed to read:

> "The coliform median MPN of the water does not exceed 70 per 100 ml., and not more than 10 percent of the samples ordinarily exceed an MPN of 230 per 100 ml. for a 5-tube decimal dilution test (or 330 per 100 ml., where the 3-tube decimal dilution test is used) in those portions of the

> area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions."

Mr. Howell, Delaware Department of Health, asked if the median coliform MPN of 70 would be changed where a 3-tube dilution was used. Mr. Lunsford replied that the change refers to the confidence limits rather than to the median value and there would be no change. Mr. Kelly confirmed that the proposed change dealt with the confidence limits. The proposal was accepted without further comment.

7. Suggested Change, Section D, Item 1, Page 20: That this item be restated to make it clear that these requirements apply equally to the interstate relaying of polluted shellfish, and that a memorandum of understanding be required between the agencies responsible for the supervision of such interstate operations.

Discussion: Shellfish production centers have changed drastically in the last few years. This change has made it economically desirable for some shellfish dealers to relay shellfish from polluted areas from one State to approved areas in another State. Such an operation could easily lead to the interstate spread of disease if the proper controls are not exercised. It is believed that the requirement of Section D, item 1 provides adequate control over relaying; however, it is not clear that these controls are intended to apply equally to interstate relaying. Also, if the controls are to be effectively carried out, a memorandum of understanding should be developed between the responsible control agencies in the producing and receiving States.

Recommendation: That Section D, item 1, be changed to read as follows:

> "Relaying--State shellfish control agencies may approve the intra or interstate transplanting of market shellfish from restricted or prohibited areas to approved areas subject to certain limitations. All phases of the operation shall be under the immediate supervision of responsible State(s) shellfish control or patrol agency(s). A memorandum of understanding shall be developed between the agencies responsible for the control of interstate relaying operations. (Shellfish may be transplanted from an approved area to another like area at any time without restriction due to sanitary reasons.)"

Section D, Item 1, "Satisfactory Compliance", Page 20:

> (g "Shellfish are not relayed intra or interstate from restricted or prohibited areas to approved areas without written permission of the State(s) shellfish control agency(s). (If shellfish are relayed interstate, a memorandum of agreement shall be developed outlining the control measures to be used.)"

Mr. Pringle, South Carolina State Board of Health, indicated that it had been their experience in the interstate shipment of oysters from polluted sources for relaying, that such memorandum of agreement was essential to satisfactory administration. He urged that the change be adopted. In response to a question from Mr. Mc K. Caldwell, North Carolina Board of Health, he also indicated that it was his opinion that such an agreement should include any State through which the shellfish might be shipped. In response to a question from Mr. Martin, PHS, Region II, he suggested that the Public Health Service assist the States in developing mutual agreements. The proposed change was, therefore, adopted without further discussion by the Workshop.

Mr. Jensen pointed out that the Workshop should go on record as either approving or objecting to the philosophy which permits the extension of relaying from an intrastate to an interstate basis. Mr. Brinsfield raised the question regarding relative merits of relaying oysters during the harvesting season and non-harvesting season. Mr. Udell and Mr. Wilbour both indicated that it was their opinion that interstate shipment of contaminated shellfish under proper supervision was acceptable. There were no further disucssions.

8. Suggested Change, Appendix B, Pages 29-32: That Appendix B be moved from Part I to Part II.

Recommendation: That this suggested change be made when Parts I and II are reprinted.

The suggested changed was adopted without discussion.

9. Suggested Change, Appendix B, Page 30: That a statement be inserted describing the responsibilities of the Public Health Service in removing the names of State certified shippers from the PHS "list" in public-health emergencies.

Mr. Jensen pointed out that the matter had been discussed at considerable length on the first day of the Workshop and it was the consensus that (1) the proposal was an infringement of State rights, (2) PHS could not exercise such authority, and (3) the Public Health Service could not knowingly permit the shipment of polluted or dangerous shellfish in interstate commerce. He noted that this was to be discussed further in regional conferences.

Mr. Turner, Florida State Board of Health, indicated that it would be his opinion that the States would not object to this authority provided the States were given the opportunity to take such action in advance of the Public Health Service. Mr, Girard strongly indicated that in his opinion this represents the infringements of States rights which would be completely unacceptable. Mr. Mc K. Caldwell and Mr. Sarraf, Pennsylvania Department of Health, indicated that they favored the proposed change.

Mr. Moorer cited their recent experience in which it became necessary to withdraw the endorsement of shippers who had oysters of questionable sanitary quality. On the advice of the PHS regional shellfish sanitation consultant, the State acted to remove the certificate.

Mr. Brown, Maryland Department of Health, also indicated that he was opposed to the system in which the Public Health Service might withdraw certification of individual shippers.

Affirmative agreement could not be reached with respect to the proposed change.

10. Suggested Change, Shucking-Packing Plant Inspection Report Form, Page 31: That the weights be changed in the following items:

Item 3, change from 1.0 to 2.0
Item 4, change from 2.0 to 1.0
Item 12, reverse the two weights.

Discussion: We question if these minor revisions justify the cost of revising the inspection forms.

Recommendation: That these items remain unchanged.

11. Suggested Change, Shucking-Packing Plant Inspection Report Form, Item 21, Page 31: That the statement "dip buckets used" be changed to read "dip buckets not used."

Discussion: This suggested change would bring this statement into agreement with the other statements found in this inspection form.

Recommendation: That the suggested change be made when the form is reprinted.

Mr. Bower, Washington shellfish industry, and Mr. Hobbs suggested that changes 10 and 11 be made when the form is reprinted. This was agreeable to the Workshop and was adopted by the Workshop without further discussion.

PART II

1. Suggested Change, Section A-2, Page 3: It is suggested that the responsibility for shell stock washing be placed on the certified dealer rather than with the harvester. Another State suggests that the full responsibility be placed on the harvester.

Discussion: It is very difficult to inspect the harvesting boats and to enforce this item if the harvester is held solely responsible. However, if the dealer is held as the responsible party, it is much easier to enforce. This problem was recognized when the manual was drafted. As now written, the harvester is required, where possible, to wash the shell stock. This assignment of primary responsibility to the harvester does not relieve the packer of responsibility.

Recommendation: No change is recommended.

A recommendation that no change be made in this item was accepted without discussion by the Workshop.

2. Suggested Change, Section A, Item 3, Page 4: That this item be rewritten stipulating that the State shellfish control agency specify the devices and practices necessary to eliminate the overboard discharge of body excretions from boats used in harvesting shellfish, and that the suggested use of soil cans be omitted.

Discussion: It is the opinion of many States that the use of soil cans is a greater public-health hazard than discharging fecal material directly into the water.

Recommendation: That item 3 be changed to read as follows:

> "Disposal of body excretions--During the marketing season, body excretions shall not be discharged overboard from a boat used in the harvesting of shellfish, or from "buy" boats while in areas from which shellfish are being harvested. The State shellfish control agency when necessary, shall specify the device and practices necessary to eliminate the overboard discharge of body excretions from boats used in harvesting of shellfish."
>
> Public-Health Reason: Gastrointestinal infections may be conveyed by shellfish; hence, it is necessary to protect the shellfish from pollution by disease-causing organisms that may be present in body excretions. This item is intended to protect the shellfish from chance pollution during harvesting. The discharging of body excretions from either harvesting or "buy" boats will be considered in the evaluation of harvesting practices.

Satisfactory Compliance, Section A, Item 3, Page 4: This item will be satisfied when:

> "a. No body excretions are discharged from a boat used in harvesting shellfish while in an area from which shellfish are being harvested.
>
> b. No body excretions are discharged from a "buy" boat while in an area in which shellfish are being harvested."

Mr. Meredith, Virginia Department of Health, inquired if devices were available which were satisfactory for the treatment of wastes from boats. Mr. Fletcher, PHS, Region III, replied that there are two different types of machines being tried out, but neither is acceptable, and that he was not aware of a satisfactory device.

Mr. Martin, PHS, Region II, indicated that there were two devices on the market which had been accepted by some States. It has been their experience, from a recreational standpoint, that they do afford a reasonable degree of protection. The States, New Hempshire and New York, are not entirely satisfied that a sterile effluent is produced, but believe that the hazard of pollution is reduced. Mr. Martin further stated the development work was being continued and devices will, undoubtedly, be developed which will afford a reasonable degree of protection. Mr. Sherman, Connecticut Department of Health, mentioned that he had recently examined several of these devices, and that in his opinion they were not fool-proof. He also noted that the chlorine supply for the units is adequate for about seven operations of the device. Mr. Brown, Maryland Department of Health, indicated that they had been working with the Department of Tidewater Fisheries in a study of the actual functioning of the units. He agreed that satisfactory operations depend on the way it is installed and maintained. He indicated that the results with installations supervised by the manufacturers in operating vessels controlled by the Tidewater Fisheries Department have not been reasonably satisfactory. He also indicated that the State was attempting to study the bacteriological effectiveness of the units, but that the project had not yet been completed. Mr. Udell, New York State Department of Conservation, explained that he had completed a two year study on the effect of pollution from small boats in marine waters. Preliminary evaluation of data, which include over 2,000 samples, has not shown a dramatic increase in pollution in marina areas. His experience with the effectiveness of the mechanical treatment devices indicates that these devices are not always effective in sewage treatment. In the absence of further discussions the proposed change was adopted as suggested.

3. Suggested Change, Section B-1, Item b, Satisfactory Compliance, Page 5: It is suggested that the words "drawn to scale on the reverse side of the certificate issued to the shipper" be deleted from both the basic item and from Item B-1. It is contended that a separate sketch or scale drawing is more practical.

Discussion: This is a practical suggestion.

Recommendation: It is recommended that the suggested change be made.

The recommended change was adopted without discussion.

4. Suggested Change, Section B-6, Satisfactory Compliance, Item d, Page 7: It is suggested that the second sentence, reading "Residual or space sprays should be used with caution to avoid contamination of the product" be deleted. It is believed that this sentence could be interpreted as indicating automatic health agency approval of these methods.

Recommendation: It is recommended that the suggested change be made.

The recommended change was agreed to without discussion.

5. Suggested Change, Section B-8, Page 7: It is suggested that the first sentence of the public-health reason be deleted and a more accurate public-health reason be developed by the Workshop.

Discussion: The present statement that working conditions impare the efficiency of the workers is hardly sufficient public-health justification for this requirement. It might also be noted that the large packing room of the Nichiro Packing Company in Hiroshima provides air conditioning in the packing room to aid in effective refrigeration of the product.

Recommendation: Further study should be made of this requirement prior to a major change in the shellfish manual. Therefore, no change is suggested.

The Workshop agreed with the above recommendation without discussion.

6. Suggested Change, Section B, Item 9 c, Satisfactory Compliance, Page 8: It is suggested that the reference to Section B, Item 10 e, be deleted at this point.

Discussion: The reference is not entirely compatible with the requirement which also appears on the same page.

Recommendation: That the suggested change be made and that the parenthetical reference be deleted.

The suggested change was adopted without discussion.

7. Suggested Change, Section B, Item 14 a, Page 10: That the statement providing for the use of galvanized returnable containers until 1960 be deleted.

Discussion: The statement is now obsolete.

Recommendation: That the following phrase be deleted from the last sentence of Item 14 a:

> "Except that the use of galvanized returnable containers in use at the time the manual was issued will be acceptable until December 1960."

The suggested change was adopted by the Workshop without discussion.

8. Suggested Change, Section B, Item 14 g, Page 11: That the item stipulate that the container rim, rather than the stand or shelf, be two feet above the floor.

Discussion: This is in accord with the real meaning of the item.

Recommendation: That Item 14 g be rewritten as follows:

> "A stand or shelf constructed of corrosion-resistant material, located so that the rim of the receiving container will be at least two feet above the floor, is provided under all chutes from skimmers and blowers, unless blowers discharge directly to a skimmer."

The suggested change was adopted by the Workshop without discussion.

9. Suggested Change, Section B, Item 14 i, and Section B, Item 24 e, Pages 11 and 18: That the construction requirement in Item 24 e be incorporated under Item 14 as paragraph i.

Discussion: It would be more appropriate that this construction item be listed under Item 14, "Construction of Utensils and Equipment."

Recommendation: That this change be made when the manual is reprinted, and that a similar change be made in the inspection report.

10. Suggested Change, Section B, Item 16, Page 12: That this item include a requirement for cleaning machines.

Discussion: Item 16 states that all equipment, utensils, and work surfaces shall be cleaned by scrubbing with water and detergent and rinsing with potable water within two hours after the day's operations have ceased. It is believed that sealing machines should be cleaned in the same manner as other equipment that comes in contact with the shucked product, and is therefore, adequately covered in Item 16.

Recommendations: That no change be made in Item 16.

It was agreed by the Workshop that no change was necessary in this item.

11. Suggested Change, Section B, Item 17, "Bactericidal Treatment of Utensils and Equipment", Page 13: It was suggested that the shucking knife and table in front of the shucker be chlorined at frequent intervals throughout the work day.

Discussion: The present requirement for bactericidal treatment of knives is compatible with that required for other equipment; however, it is not compatible with the requirement of Section B, 16 g, which states that blowers and shucked-stock holding containers be flushed or spray rinsed with tap water after each emptying nor with the requirement of Section B, 21, e, which stipulates that shucking containers shall be rinsed with running tap water before each filling. It would appear that an excellent argument could be made for more frequent sterilizing of the knives and shucking tables; however, we do not believe that such a change should be recommended unless bacteriological studies of shucking procedures confirm to this need.

Recommendation: No change is recommended. However, it should be emphasized that shucking knives are to be considered as part of the utensils and equipment, and that they are to be subjected to an effective bactericidal process at the end of each day's operation.

The Workshop agreed that this suggested change was not necessary.

12. Suggested Change, Section B, Item 21 f, Page 16: That a definite size be specified for shellfish shucking buckets and pans as in the proposed equipment construction guides.

Discussion: The requirement of the manual pertains to the length of time that shucked shellfish are allowed to remain on the shucking bench. The time required to shuck a gallon of shellfish varies considerably depending upon the species of shellfish, e.g. It would not be appropriate to specify the same size of container for the Olympia oyster as for the Pacific oyster, since it takes approximately 2,500 Olympia oysters to make a gallon as compared to only 64 Pacific oysters. Therefore, we believe that it should be the State's responsibility to specify a container size that will meet the two hour limitation.

Recommendation: That no change be made in this item.

It was agreed without discussion by the Workshop that no change should be made in this item.

13. Suggested Change, Section B, Item 24 f, Page 18: That a sentence be added to permit returnable shipping containers to be labeled with tags.

Discussion: In a few States, returnable containers are used to ship shucked shellfish to repackers. It has been suggested that the earlier requirement for permanent shipper identification is not practical with the expensive aluminum and stainless steel containers now in use.

Recommendation: That the following sentence be added to Item 24 f, Page 18:

> "Returnable shipping containers may be identified with tags of at least 2 5/8 x 5 1/4 inches in size and made of substantial water-proof stock."

Mr. Wallace inquired if the change was to be interpreted as meaning that the identification of returnable shipping containers by use of a shipping tag was an alternate to the present system of embossed names and numbers. Mr. Jensen affirmed that it was. Mr. Wallace then questioned the phrase "It is not practical with expensive aluminum or stainless steel containers", noting that suppliers could emboss the names of the purchasers. Mr. Hobbs, Maryland Department of Health, indicated that this was impractical. He explained the containers were frequently owned by the repackers rather than the initial shucker and that it was more practical to use the tags. Mr. Gregory, Virginia Department of Health, also indicated that repackers in Virginia had purchased large number of containers for use by the plants which supply them shucked oysters. The use of permanent identification on containers results in continual mix-ups as to the true identify of the products. He went on to suggest that a special tag, smaller than the 2 5/8 x 5 1/4 size now used for shell stock, be developed. Mr. Jensen indicated that PHS would initiate the development of such an identification tag.

In the absence of further discussion, the change was accepted.

14. Suggested Change, Section B, Item 25 a, Page 18: It is suggested that cooling to a temperature of 40°F. temperature be required.

Discussion: Available information suggests that from quality control standpoint, it would be desirable for the shellfish industry to adopt this 40°F. temperature requirement; however, there seems to be no public-health justification for such a requirement. We do not believe that a "public-health lever" should be used to force the industry to adopt what may seem to be better quality control practices. It must however, be recognized that the industry is represented in the administration of the Cooperative Program and that such a change could be made with industry concurrence.

Recommendation: It is recommended that no change be made in the requirements for refrigeration temperatures for shucked shellfish.

The proposed change was not accepted by the Workshop.

15. Suggested Change, Section C, Table II, Page 21: It is suggested that Table II be revised to indicate those items in Sections A and B which are applicable to each type of shell stock shipping and packing operation.

Discussion: Table II lists the items that are applicable to all types of shell stock operations. Experience with the manual suggests the need for a more explicit listing of requirements.

Recommendation: That footnote #18, Page 21 be eliminated; that the following Table replace Table II, Page 21; and that the third paragraph, Section C, Page 21 be changed to read as follows:

> "To effectuate the needed sanitary safeguards, the shell stock shipper shall comply with Item 1 and 2 below, and with the Items of Sections A and B indicated for each type of shell stock operation in Table II."

That the following sentence be added to Section C-2, Page 21:

> "The following classes of shell stock shippers shall be exempt from this requirement: Harvesting Only; Buy Boats; and Buy Boats with Storage on the Boats."

Mr. Jensen indicated that the present Table II in Part II, shellfish manual, had not been sufficiently explicit, and that representatives of the New England and Middle Atlantic States, and the Region II shellfish sanitation consultant, had developed the substitute Table II below:

TABLE II

Type of Shell Stock Operation	Shore Establishment Required	Item Number in Section B 1	3	9	10	11	12	15*	19	20	27	28	Comments
Harvesting Only	-	-	-	-	-	-	-	x	x	x	x	x	No Storage
Harvests & Packs	-	-	-	-	-	-	-	x	x	x	x	x	Packing On Boat
Harvests, Stores & Packs	x	x	x	x	x	x	x	x	x	x	x	x	Storage Ashore
"Buy" Boat	-	-	-	Sec. A	-	Sec. A	-	x	x	x	x	x	
"Buy" Boat & Storage	-	-	x	Sec. A	-	Sec. A	-	x	x	x	x	x	Storage Only On Boat
"Buy" Truck	-	-	-	Sec. A	-	-	-	x	x	x	x	x	
"Buy" Truck & Storage	-	-	x	Sec. A	-	-	-	x	x	x	x	x	Storage On Truck
Establishment Buy, Packs, & Stores	x	x	x	x	x	x	x	x	x	x	x	x	
Establishment Buy & Store Ashore	x	x	x	x	x	x	x	x	x	x	x	x	

X = Required
- = Not Required

* Part II, Section B, Items 15 (b) and 15 (f) are not applicable to shell stock shippers.

The proposed change was adopted without further discussion.

16. Suggested Change, Section C, Item 2 b, Page 22: That a sentence be added to require shell stock shipper tags to bear information as to the local waters from which the shellfish are taken.

Discussion: Because of previous unsatisfactory experience, a similar requirement was dropped from the manual at the time of the 1957 revision. In practice it has been found that only a general area identification such as Chesapeake Bay, Miles River, or Delaware Bay were given. The loss of identify was further complicated by the common practice of intermingling shell stock from various dealers and sources. We believe that the inclusion of a specific shell stock identification requirement could have meaning only if drastic changes were made in industry harvesting and handling procedures. Such identification is, in many instances, essential in making epidemiological investigations of shellfish-associated disease outbreaks. Until recently, we had been of the opinion that such information was needed so infrequently that (1) we were not justified in requiring industry to go to great expense in keeping such records, and (2) that the system would be so complex that it would be administratively unsound. The recent experiences in Mississippi and New Jersey suggest that this position be reexamined; however, these are practical considerations which cannot be ignored, e.g., the use of "buy" boats in Chesapeake Bay and the frequent intermingling of hard clams in the New Jersey-New York area.

Recommendation: That this item remain unchanged; however, State inspectors should emphasize the importance of meeting existing record requirements.

Mr. Udell referred to the experience of the past year with epidemiological investigations of shellfish associated with infectious hepatitis in which difficulties had been experienced in identification of shell stock shipments. He recommended that shell stock tags show the specific area from which shellfish were harvested, and the date the original shipment or date of packing of the shell stock. He pointed out that the present requirement for information as to the consignee, is useless and impractical as the original shipper may have no information as to the eventual consignee. Mr. Macomber pointed out that it was the unanimous opinion of all States in Region I and II (from Maine to Delaware) that this change should be made. Mr. Fortgang, New York City Health Department, also went on record as favoring the changes.

Mr. Brown, Maryland Department of Health, agreed with the previous speakers as to the desirability of better identification for shell stock shipments. He pointed out this requirement would pose some serious administrative problems with buyboats which purchase shellfish from many different areas, but that mechanisms could be developed which would indicate the source of shellfish and the given days of harvesting or shipment. Mr. Gregory was in agreement with Mr. Brown. Mr. Mc K. Caldwell, North Carolina State Board of Health, expressed a similar position. He added that identification of shell stock was not a major problem in North Carolina because only hard clams are shipped as shell stock. Mr. Pringle, South Carolina Board of Health, and Mr. Cook, Florida State Board of Health, agreed that information of this type was necessary.

Mr. Dean, Georgia State Board of Health, described the Georgia shell stock tag which requires information regarding the source. Mr. Howell, Delaware State Board of Health, explained that Delaware law requires source information, but pointed out that the requirement is difficult to enforce and that he questioned the accuracy of some of the information which was recorded on the tag.

Mr. Spath, Louisiana State Board of Health, stated that Louisiana law requires source information. He noted that dealers at times show a bed number in preference to the local name of the water. Mr. Moorer, Mississippi State Board of Health, indicated that State law requires source information on shell stock tags. He also noted the use of bed numbers as described by Mr. Spath. Mr. Griffith, Alabama Department of Health, stated that Alabama required this information on the tags and on a ledger to be maintained by the plant.

Mr. Lauderdale, Texas State Health Department, stated that Texas law required identification of oysters produced in Texas. He noted that few shell oysters were exported from Texas. Mr. Blair, Oregon State Board of Health, stated that Oregon laws required tagging and that it was his understanding that Washington also required this information.

Mr. Wallace indicated that he was in agreement with the need for identity of source of shell stock, but seriously questioned if it would be possible to obtain accurate labeling of sources of shell stock.

Mr. Jensen summarized by stating that there was agreement that shell stock should be positively identified, and that the consignee's name should not be required. He indicated that he appreciated the problem of how best to define sources and suggested that it be taken up on a regional basis. He also suggested that the date on which this requirement would not be put into effect until after regional discussions.

Mr. Gregory pointed out that the New York City requirement for a shell stock identification tag (perforated tag) is different from that of the shellfish manual, and suggested that the requirements be brought together. Mr. Jensen indicated that this would be referred to the regional shellfish consultants, and that in New York City, Mr. Macomber would be responsible for making the necessary arrangements for discussing this problem with Mr. Udell and the New York City authorities.

Mr. Brown again pointed to the practical problems of obtaining effective identification of shell stock sources and urged that the need for actual identification rather than a mere recording of "source" be recognized. Mr. Jensen replied that it was this very consideration that led to the 1956 change in the shellfish manual which deleted the requirements for source identification; that is, specific information was not obtainable. Mr. Martin, PHS, Region II, indicated that uniform guidelines would be necessary to assist the regional offices in their evaluations of State shellfish sanitation programs.

Mr. Griffith stated that it was his belief that the outbreak early in 1961 placed many of the State programs in jeopardy and that it was industry's responsibility to know where the shell stock came from. Records should be sufficiently complete to permit an identification of the general area from which the shellfish were harvested. Mr. Jensen then suggested that industry also be represented on the discussions of the means for better tagging of shell stock shipments.

17. Suggested Change, Section C, Item 2 b, Page 22: That a sentence be added to explain what is meant by bulk shipment.

Discussion: Some packers have interpreted this to mean that large shipments of shell stock, even though packaged, (bags, boxes, or baskets), are bulk shipments, and therefore require only one tag. Experience has shown that this practice results in poor identification of the individual packages and defeats the purpose of the requirement.

Recommendation: That the last sentence in Item 2 b be modified by inserting "e.g., unpackaged" after the word "shipments."

Mr. Wallace said that this had been a problem in recent years where bulk shipments were transported from the Gulf Coast to packers in the Middle Atlantic and Chesapeake Bay areas. Shippers in the Gulf area have complained that since oysters are consigned to one packer, they believe it should not be necessary to have identification tags on each package. Mr. Jensen indicated that, from a theoretical standpoint, he was in agreement with this position and pointed out that in 1956 the shellfish manual was revised to permit the use of a single invoice with such a shipment. From a practical standpoint, it has been found that such consignments were frequently broken up and that sub-shipments without tags lost their identity. Mr. Gregory noted that not all the bags of oysters are sealed and questioned if a bag of oysters, which was not closed, would actually be considered packaged. Mr. Jensen replied that it would be.

The proposed recommendation was accepted without further discussion.

Proposed Amendment to Section 27, Part II:

Mr. Goggins, Maine Department of Sea and Shore Fisheries, commented on the need for industry records which would help to identify the product and which would provide statistics useful in fisheries management programs. Mr. Turner, Florida State Board of Health, noted that the State Department of Conservation and Fish and Wildlife Service collected such statistics in Florida and that these could be obtained through Fish and Wildlife Service. Mr. Glude, Fish and Wildlife Service, explained that the FWS has a statistical service which was organized to obtain the type of statistics which could be used by State agencies in fisheries management.

The subject was not resolved, however, it was agreed that better record keeping systems were needed and that meetings should be held with industry members to work out suitable plant systems for record keeping.

Mr. Lauderdale, Texas Department of Health, informed the Workshop that Texas regulations require that each shipper submit monthly, a daily record of shipments and purchases, noting that the shippers had been relatively cooperative in complying with this requirement. Mr. Mc K. Caldwell, North Carolina State Board of Health, stated that North Carolina shippers kept records for tax purposes, and that they were open to inspection by State personnel as needed.

Mr. Goggins proposed that Section 27 be revised to read "Complete and accurate records shall be kept by every shellfish dealer and sent weekly to a designated control agency on an appropriate record form." Mr. Jensen indicated that he was in agreement with Mr. Goggins's suggestion that plant records were not generally satisfactory, and suggested a meeting with Mr. Wallace and others in the oyster industry to develop a record system which would fulfill the needs of the certification program without being to burdensome on the industry.

Mr. Stringer, Fish and Wildlife Service, indicated that he agreed that fisheries management required good records, but he was not prepared to give an official opinion as to the adequacy or inadequacy of existing records. Mr. Wallace indicated a similar position.

Wednesday Afternoon - November 29, 1961

Dr. Arthur Novak, Chairman

Results Obtained in the Collaborate Study of the Sanitary Significance of the E. Coli in Shellfish Growing Areas:

Mr. Jensen opened the discussion by tracing the history of the cooperative investigation. In 1950, the States, the Public Health Service, and the Government of Canada undertook a collaborate study of the bacteriological quality of shucked Eastern oysters at the market level. Early findings suggested that coliforms were not satisfactory indicator organisms for this purpose and extended study of other indicators was undertaken. Preliminary results suggested that the Eijkman method for the enumeration of *E. coli* would be the most satisfactory of these several possible tests. At the 1958 Workshop a tentative bacteriological standard for shucked oysters at the market level based on the *E. coli* (Eijkman) MPN and Standard Plate Count was accepted. It was evident to the participants in the 1958 Workshop that this standard would need to be related to conditions in the growing areas. The 1958 Workshop therefore requested that the PHS organize a cooperative study with the States to study E. C. levels in widely separated geographic areas. Mr. Jensen indicated that the following series of papers described the progress made in this study.

Mr. Joseph A. McCarthy, Chief, Lawrence Experiment Station, Massachusetts Department of Health, presented a paper entitled "Collaborative Study of Sanitary Significance of E. C. Positive Organisms in Shellfish Growing Waters." Mr. McCarthy's paper is included as Appendix FF of these Proceedings.

Mr. Robert Brown, Chief, Bureau of Environmental Hugiene, Maryland State Department of Health, presented a paper entitled "Coliform and E.C. Determinations." A copy of Mr. Brown's paper is included as Appendix G of these Proceedings.

Mr. J. A. Hope, Bacteriologist, Virginia State Department of Health, presented a paper entitled "Coliform E. C. Gas Positive Relationships in Shellfish, Seawater, and Bottom Sediments From Areas of Varying Bacterial Quality". A copy of Mr. Hope's paper is included as Appendix H of these Proceedings.

Dr. Thomas S. Hosty, Alabama State Health Department, presented a paper entitled "The Significance of EC Positive Organisms in Gulf Shellfish Growing Waters". A copy of this paper is included as Appendix I of these Proceedings.

Mr. C. B. Kelly, Chief, Shellfish Sanitation Laboratory, Purdy, Washington, presented a paper entitled "Sanitary Significance of 'Fecal' Coliform Organisms in a Shellfish Growing Area; Sanitary Survey of Burley Lagoon". A copy of Mr. Kelly's paper is included as Appendix Z of these Proceedings.

Dr. A. D. Tennant, Canadian Department of National Health and Welfare, presented a paper entitled "Confirmation Test for the Estimation of *Escherichia coli* Densities in Seawater and Shellfish". A copy of this paper is included as Appendix J of these Proceedings.

Mr. Thomas P. Blair, Oregon State Board of Health, presented a paper entitled "Sanitary Survey of Tillamook Bay and Sanitary Significance of the 'Fecal' Coliform Organisms in Shellfish Growing Waters". A copy of this paper is included as Appendix K of these Proceedings.

Dr. Joel Kaplovsky, Director, Delaware State Water Pollution Commission, presented a paper entitled "Some Aspects of Cause and Effect Relationship Pertaining to Water Quality in Estuarine Areas". A copy of Dr. Kaplovsky's paper is included as Appendix L of these Proceedings.

Dr. Novak thanked the participants and opened the subject to discussion from the floor.

Dr. Slocum noted the tremendous amount of effort that had gone into the study, and suggested that it had produced much badly needed information. He explained that the Food and Drug Administration has been interested in the E. C. test for use with other foods, but has found that it is. apparently, not always as satisfactory as it has been with shellfish. He noted that 65 to 70 percent of the gas positive tubes will actually confirm as E. coli types I and II. They have found that an incubation temperature of 45.5°C. is more critical in separating E. coli from other coliform types and gas production at this temperature, more accurately indicates the presence of E. Coli. Out of a set of 2,600 tubes incubated at 45.5°C., 93 percent confirmed as E. coli types I and II.

Mr. Siegel,Strasburger and Siegal,*/ raised a series of questions concerning the bacteriological standards which might be applied to shucked oysters, pointing out the administrative difficulties in using the present interim standards. Mr. Jensen and Mr. Wallace pointed out that the subject of market standards was not under consideration at this point and suggested that Mr. Siegel comments be withheld until after Mr. Kelly's review of results obtained in the past three-year study of the interim standards.

Dr.Kaplovsky noted that there was an inverse correlation between coliform counts and water temperature and that coliform counts tend to be lower in summer than in the winter. He, therefore, raised a question about the times at which samples had been collected, noting the fact that coliform standards were met during the summertime did not necessarily indicate the same standards could be met during the winter. He pointed out that Mr. Blair had reported that periods of light run-off had lower counts than high run-off periods and asked if there might not also be a temperature factor.

Mr. Kelly replied that the area which Mr. Blair discussed has about 100 inches of rainfall per year and that the area in which he had conducted his studies, Burley Lagoon, had about 30 inches of rainfall. However, during the periods of May 30 to July 30, there was a seven-week period without measureable rainfall. This is at variance with the situation reported by Dr. Hosty with periods of extremely heavy rainfall and run-off. He indicated that it was his impression that this was a geographical condition, and he stated that it was his opinion that the papers suggested that, in many situations, the E. C. test offered a better method of evaluating levels of pollution than the simple coliform test.

Mr. Jensen then raised the question as to how the findings of the cooperative study might be applied to the Cooperative Program. The Chairman, Dr. Novak, suggested that Mr. Kelly appoint a group of individuals to evaluate the papers which had been presented and develop recommendations which could be considered by the Workshop.**/

Sanitary Significance of Radionuclides in the Marine Environment from the Standpoint of Shellfish Sanitation:

Dr. J. E. Campbell, Public Health Service, presented a paper entitled "Radionuclides in the Marine Environment". A copy of this paper is included as Appendix M of these Proceedings.

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*/ Strasburger and Siegal, Consulting Bacteriologists, Baltimore, Maryland. The firm has been active in shellfish bacteriology for many years.

**/ Mr. Kelly, subsequently, reported that a committee to consist of Dr. Novak, Mr. Jensen Dr. Tennant, Mr. McCarthy, Mr. Wallace, Dr. Hosty, Dr. Slocum, and Dr. Perry would review the material presented at the Workshop and to develop recommendations which could be later presented to all interested parties for comment.

Thursday Morning, November 30, 1961

Mr. John Glude, Chairman

Review of Results Obtained in the Collaborative Study of Bacteriological Quality of Shellfish in Eastern Oysters:

Mr. C. B. Kelly, Chief, Shellfish Sanitation Laboratory, Purdy, Washington, presented a paper entitled "Results of Interstate Collaborative Study to Evaluate Interim Bacteriological Standards for Shucked Oysters". A copy of Mr. Kelly's paper is included as Appendix N of these Proceedings.

Dr. Hosty inquired of Mr. Kelly if the fidelity of the E. C. test with oysters compared reasonably well with that which was found with water. Mr. Kelly replied that it did not. Working with fresh oysters, approximately 85 percent of the E. C. gas positive tubes were found to be positive for E. coli type I; however, with storage, the fidelity declined, reaching a level of about 80 percent. He indicated that it was his understanding that the work in the State of Washington, not yet reported, obtained a fidelity of approximately 91 percent.

Mr. McCarthy reported a fidelity of approximately 82 percent working with soft clams. Mr. Kelly replied that he agreed with Dr. Hosty's suggestion that a more consistent test for a particular group of organisms was highly desirable. Dr. Hosty replied that it might be well to consider Dr. Slocum's suggestion that an increased temperature of one-half degree might narrow the spectrum considerably.

Mr. Spath, Louisiana State Board of Health, stated that oysters produced in Louisiana could not consistently meet the interim standards and urged that they be liberalized. Mr. Kelly countered with the statement that the data submitted by Louisiana did not indicate that their production did not meet the standards. He explained that the standards were not intended to apply to single samples and they called for investigations rather than for confiscation.

Mr. Wallace, Oyster Institute of North America, urged that the Workshop reach some decision with respect to the standards--continue along as an interim standard, or adoption as a final market standard, or abandonment. He pointed out that the data presented in the study indicated that the industry cannot always meet the limits of the interim standards, citing Table II of Mr. Kelly's paper as evidence. Mr. Kelly referred to the discussions which had taken place at the earlier 1958 Workshop in which both Mr. Kelly and Dr. Harris had urged the adoption of higher limits. He again reiterated that the standards were not intended to apply to individual samples and that they called for investigation rather than confiscation.

Mr. Wallace replied that from a practical standpoint, the interim standard would not be so used, and cited a case which occurred in Chicago in the fall of 1961where a number of Chesapeake Bay packers were stopped from shipping to Chicago on the basis of failure to meet the interim standards. He indicated willingness to accept the present standards if there could be assurance that they would be correctly used, but questioned if this could be accomplished. Mr. Van Derwerker, PHS, Region V, suggested that interior States and municipalities were going to adopt bacteriological standards and agreed that action such as Mr. Wallace had described had been detrimental to the industry. He then noted that it was his understanding that the shippers which were denied access to the Chicago market were not from the Chesapeake Bay area and that they were excluded only after three consecutive samples were reported to have E. C. values greater than 230. He added that this action had been reported in the newspapers and this had undoubtedly hurt both the shipper and the State. While such actions are unfortunate, he suggested that similar occurrences may be expected, and this has furnished evidence as to the need for the Workshop to take positive specific action and for the PHS to give States and muncipalities information which should be included in ordinances. The alternative to a uniform standard is a hodge podge of standards which would result in chaotic conditions in shellfish receiving areas.

In response to a question from Mr. Howell, Delaware State Board of Health, Mr. Kelly explained that the interim standards were intended to apply specifically to shucked Eastern oysters at the market level.

Mr. Jensen defended Mr. Wallace's stand suggesting that experience indicated that control agencies would fail to use the standard correctly, that is, it would be applied to oysters other than the shucked Eastern oysters, to oysters which had been frozen, to single samples, and to oysters of unknown sanitary quality.

Mr. Brown stated that it had been their experience that the interim standard had demonstrated its worth, and that Maryland shippers could operate within its framework.

The Chairman, Mr. Glude, summarized by pointing out there seem to be complete agreement on the philosophy of a bacteriological standard for shucked oysters, but that there was disagreement as to the values which might be used in such a standard. He suggested the formation of a small committee to review the data and to develop recommendations.

Mr. Fortgang, New York City Health Department, indicated that it was his belief that there was need for an acceptable uniform standard, and expressed a hope that the Workshop would be able to develop a workable bacteriological standard for oysters received at the market. He noted that New York City has used a coliform MPN standard of not more than 2,400 per 100 ml. The City recognized the work that was being undertaken in this field and could revise their standards to agree with those that might be recommended by the National Shellfish Sanitation Workshop. He agreed that the coliform group has not proven to be a meaningful indicator of contamination. Administratively, the current interim standard has proven to be a useful tool and he expressed hope that a permanent standard might be adopted. He noted that the interim standard had greatly reduced friction between his Department and the producing States, and that the States had cooperated freely with the City in the investigation of shippers having unusually high coliform or E. C. levels.

Dr. Novak noted that the E. C. standards which had been adopted were based on levels which might be obtained by industry rather than on those that might have health significance. He asked if there were any figures available which would show the levels of pollution which might be necessary for health hazards. He urged that the Workshop not take action to formally adopt an interim standard until it can be associated with health hazards. Mr. Kelly replied that it was his feeling that the operating experience of the Cooperative Program over a long period of time did not show a record of associated enteric disease with commercially produced shellfish. He suggested that use of the interim standard had produced oysters of satisfactory quality rather than oysters which were of a potential health hazard, and that in his opinion, it was satisfactory although some readjustment of limits might be necessary. He noted that the standards were intended to apply only to oysters of known sanitary quality and not to oysters from an unknonwn source.

Dr. Novak indicated concurrence with Mr. Kelly's remarks and pointed out that this confirmed Mr. Wallace's earlier contention that the bacteriological quality of shellfish, as now marketed, is satisfactory as measured by the absence of disease transmission and that interim standards should not reject a certain portion of this current production. Dr. Novak further noted that it is a recognized fact that the present methods of measuring microbiological quality do not include tests for the presence of pathogenic virus, rickettsia, yeasts, molds or amoeba.

Mr. Glude, Chairman, proposed that Mr. Kelly be directed to form a committee to work with him in a review of the data, and to make recommendations for future use of the interim standards.

Review of Construction Guides for Shellfish Handling and Packaging Equipment:

Mr. Lee Roy Lunsford, Public Health Service, had prepared a paper entitled "Equipment Construction Guides for Shellfish Handling and Packing Equipment" for presentation at the Workshop. However, at Mr. Lunsford's suggestion to conserve time, Mr. Jensen briefly discussed the purpose and use of the Equipment Construction Guides which had previously been circulated for review and comment. Mr. Jensen further indicated that the comments received had been generally favorable.

Mr. Hobbs stated that it was his opinion that the placement of the tanks two feet above the floor would make them too high to permit the dipping of oysters. Mr. Jensen pointed out that the overall height of the tanks was stated to be not more than 40 inches with a 12 inch minimum clearance to allow space for cleaning. The guide was adopted by the Workshop without further discussion.

Chromate Test for Oyster Freshness:

Dr. Mary Schafer, Public Health Service, Cincinnati, and Mr. John Hope, Virginia State Department of Health, presented a paper entitled "The Chromate Color Test". A copy of this paper and a paper entitled "Report on the Cooperative Field Study on the Chromate Color Test for Determination of the Time-Temperature History of Raw Shucked Oysters" are included as Appendix O of these Proceedings.

Mr. John Hope then reviewed several experiments conducted with the chromate test on oysters. A copy of Mr. Hope's discussion is included as Appendix P of these Proceedings.

Public-Health Significance of Industrial Waste of Shellfish Growing Areas:

Dr. Leonard Kurland, National Institutes of Health, Epidemiology Branch, presented a paper entitled "Minamata Disease". A copy of Dr. Kurland's paper is included as Appendix Q of these Proceedings.

Dr. Kurland illustrated his paper with a series of slides and a movie showing the investigations which were made in Minamata Bay, Japan. Mr. Bower referred to Dr. Kurland's presentation as proof that the PHS and the States should be concerned with wastes of industrial plants and with other chemicals such as those which might be used for the control of predators. He also suggested that industrial wastes could influence coliform or E. coli findings in growing areas and in shellfish.

Mr. Harris, Louisiana State Department of Health, noted that major industrial developments are located along the Louisiana and Texas coasts. He asked Dr. Kurland which industries in the Galveston area were major contributors of this type of pollution. Dr. Kurland replied that to his knowledge the only source of the toxic agent is in vinyl chloride production. He noted that in Minamata Bay, the danger was from loss of a catalyst rather than the organic compounds used in manufacturing. He stated this was perhaps the reason that it took so long to determine the cause of the disease. Most people think of catalysts as remaining in the reactor system, however, in the Minamata case, quantities of the catalyst were discharged with the wastes. He reported that the Galveston area had four plants making vinyl chloride. He explained that industry was reluctant to release production figures, but that it was believed that over 100,000 tons of vinyl chloride was produced by the four plants, approximately half was produced usingthe mercuric chloride system, so that something in the order of 2,000 kilograms of mercuric chloride would be lost. However, only a small fraction of this mercury was being discharged into Galveston Bay, since the plants distill this vinyl chloride product and mercuric oxides are dispersed into the air rather than into nearby waters.

Mr. Harris noted that a number of petrochemical industries have plants in Louisiana and produce similar compounds. Mr. Glude stated that it was his opinion that this report demonstrated the need for the health agencies to determine any possible sources of danger, and that such action was needed both the public-health protection and for the economic protection of the industry.

Thursday Afternoon - November 30, 1961

Mr. Robert Brown, Chairman

Continuation of Discussions of Public-Health Significance of Selective Industrial Wastes:

Mr. McFarren presented a paper entitled "The Occurrence of Copper and Zinc in Shellfish". A copy of Mr. McFarren's paper is included as Appendix R of these Proceedings.

Dr. Kurland asked Mr. McFarren if he had investigated the possibility that a protein material might be responsible for the animal deaths in the bioassay, and if there was any information as to the possible sources of the zinc and copper in the shellfish. Mr. McFarren replied that experience with the use of this test for paralytic shellfish poison did not indicate that protein material would be harmful to the mice. He also noted that some of the samples of oysters had been ashed, and that this material, which would not contain proteins, was toxic to mice. In response to Dr. Kurland's second question, Mr. McFarren replied the matter was under investigation but that he had no information to report. Mr. Brown, Maryland State Department of Health, replied to Dr. Kurland. He indicated that the State had no information which had shown that the zinc and copper could be traced to industrial waste discharges, and that they had obtained assistance from Johns Hopkins University, Chesapeake Bay Institute, to investigate the problem. He reported that a special meeting of State-PHS personnel would be held in the near future to review this matter. Dr. D'Aguanno, Food and Drug Administration, asked if comparative figures were available which would indicate a trend in the level of concentrations. Mr. McFarren replied that all available data was included in the tabulations. Dr. D'Aguanno then asked if the data was based on dry weights. Mr. McFarren said it was based on the normal wet weight of the oysters.

Public-Health Hazards in the Use of Chemicals for Control of Shellfish Predators:

Dr. Victor Loosanoff, U. S. Shellfisheries Laboratory, Milford, Connecticut, presented a paper entitled "Progress in Development of Methods of Chemical Control of Shellfish Predators". A copy of this paper is included as Appendix S of these Proceedings. Dr. Loosanoff's presentation included a color movie of the experimental use of such chemicals in shellfish predator control. In concluding his presentation, Dr. Loosanoff pointed out that the Fish and Wildlife Service now has a contract with the U. S. Testing Cooporation, Hoboken, New Jersey, for the development of analytical procedures of the material in oysters.

Mr. Shaw, Massachusetts Department of Natural Resources, asked if there was any information as to how the material might effect bay scallops. Dr. Loosanoff replied that there was no direct experimental evidence, but that experiments with other bivalves indicated that scallops would not be effected. Dr. Loosanoff added that he hoped that it would be possible within the next year to obtain an official release for use of the material.

Mr. Homans inquired as to the knowledge of the physiological processes involved. Dr. Loosanoff replied that this had not been investigated, and noted that his laboratory staff was very small and that they had not been able to undertake studies of this nature.

Mr. Robert S. Roe, Director of Bureau of Biological and Physical Sciences, Food and Drug Administration, presented a paper entitled "The Food and Drug Administration Viewpoint". Mr. Roe's paper is included as Appendix T of these Proceedings.

Mr. Bower, Washington shellfish industry, inquired as to the scope of Food and Drug Administration law with respect to chemicals used for predator control. Mr. Roe replied that Food and Drug Administration was interested in food contamination with any substance that is poisonous or deleterious. He noted that in the case of deliberate application, as with pesticides and food additives, the law has set up procedures for establishing tolerances or regulations for their safe use. Another section of law, applicable in the case of contaminants, considers food as adulterated if it contains any added harmful substances unless such substances are necessary in the production of the food or cannot be avoided by good manufacturing practice. In such cases the law gives authority to set regulations or tolerances, if such can be set.

Mr. Jensen explained that the PHS has no direct legal responsibility in this matter, but in line with responsibilities under the Cooperative Program, the Service would expect to work with the States and the shellfish industry to develop methods for the safe utilization of these materials accepted for use by FDA.

Mr. Roe further added that the Food and Drug Administration has appreciated the interests of the PHS and the Fish and Wildlife Service in these technical and scientific problems, and it is hopeful of pooling information and ideas.

Mr. Glancy, New York shellfish industry, noted that New York State shellfish industry has had great difficulties with predators and was hopeful that chemical control methods could be developed in the relatively near future. Mr. Wallace enlarged on this, pointing out that both the Chesapeake Bay and West Coast areas had predator problems which might be controlled by chemical methods. Both Mr. Mears, Chesapeake Bay Oyster industry, and Mr. Bower, Washington State oyster industry, agreed with Mr. Wallace's statement.

Dr. Loosanoff estimated that in the United States effective chemical predator control might result in saving of 50 million dollars worth of oysters a year. He cited the ghost-shrimp problem on the West Coast where chemical control measures costing about $35 per acre are nearly 100 percent effective against this animal which has destroyed hundreds of thousands of acres of oyster beds.

Mr. Eugene Jensen, Chief, Shellfish Sanitation Branch, presented a resume of a paper entitled "Conditionally Approved Areas". Mr. Jensen's complete paper is included as Appendix B of these Proceedings. (Mr. Jensen's entire paper could not be presented because of time limitations.)

Paralytic Shellfish Poison Situation in the United States and Canada:

Dr. A. Prakash, Fisheries Research Board of Canada, presented a paper entitled "Status of Paralytic Shellfish Poisoning Research in Canada". Dr. Prakash's paper is included as Appendix V in these Proceedings.

Mr. Phillip Goggins, Maine Department of Sea and Shore Fisheries, presented a paper entitled "Paralytic Shellfish Poison". Mr. Goggins' paper is included as Appendix W in these Proceedings.

Dr. A. K. Sparks, University of Washington, presented a paper entitled "Status of Paralytic Shellfish Toxicity Studies in Washington". Dr. Spark's paper is included as Appendix X of these Proceedings.

Dr. Loosanoff referred to the apparent relationship between nitrate in the water and the development of toxicity and questioned if this toxicity might not be due to the increased feeding activities of the mollusks. Dr. Sparks replied that there were several possibilities. For example, the increase in temperatures and nitrates permitted the flagellates to multiply rapidly; and, simultaneously, the shellfish would begin to feed more rapidly. Secondly, the nitrates may have dropped off because they were taken out of the water by the dinoflagellates by the process of multiplication. He stated that the data was not sufficient to support any conclusions at this time.

Mr. Earl McFarren, PHS, Cincinnati, presented a paper entitled "Present Status of the Paralytic Shellfish Poison Problem". A copy of Mr. McFarren's paper is included as Appendix Y of these Proceedings.

On conclusion of Mr. McFarren's paper, the Chairman opened the subject for general discussion. Mr. Esveldt asked why Dr. Sparks did not include razor clams in his studies. He noted that at least 400,000 diggers last year took over 8 to 12 million pounds of clams, and that the hydrographic conditions on the coast are much different than those of Puget Sound.

Dr. Sparks replied that the toxicity levels had been checked in some razor clams; however, they were not included because the toxin is generally concentrated in the digestive system which is removed. Mr. Girard confirmed Dr. Spark's statement. He noted that bioassay of the entire clam will show a level of approximately 200 mouse units. A bioassay of the digestive tract of these clams would show four to five thousand mouse units with the remaining edible portion of the clam testing negative.

Mr. Jensen, asked if Dr. Sparks had calculated the conversion factor from mouse units to micrograms. Dr. Sparks replied that their studies had not reached a point where they were concerned with absolute measurement, and it had not been necessary to calculate the conversion factor. He indicated that his source of mice was the same as the Washington State Department of Health and that he assumed that conversion factor would be about the same as the State's.

Mr. Homans noted that people along coastal areas and who frequently eat shellfish, may develop a tolerance for the poison. He cited an incident in Nova Scotia where the local people eat fairly large quantities of toxic mussels and clams without suffering apparent ill-effects.

Dr. Sparks cited an experience with a local clam digger living near the entrance to Sequim Bay, Washington. Toxicity levels for clams in this area average 2,000 to 1,500 mouse units. The clam digger informed Dr. Sparks that he ate the clams year round and said that he could tell when the "red tide" was in because his fingertips, toes, and ends of ears would tingle, and added that he lost most of his cats in the summertime.

Mr. McFarren, SEC, PHS, also indicated that there is evidence that some people may develop a tolerance for the poison.

Dr. Loosanoff pointed out a need to cultivate the organisms.

Dr. Prakash indicated that they have succeeded in isolating and culturing the toxin producing dinoflagellate in the Bay of Fundy and were using the culture methods developed by Provasoli. Preliminary laboratory investigations have indicated that (1) enriched sea water and ASP7 (an artificial medium with Vitamin B-12) are most suitable for growing mass cultures, (2) non-toxic shellfish become toxic if fed on these cultures, and (3) the growth of the dinoflagellates is significantly affected by minor changes in temperature, light intensity, and nutrients. He noted that toxicity predictions for growing areas would eventually be based on environmental conditons which would, among other things, involve measurements of nutrients, solar radiation, and temperature.

Dr. Loosanoff agreed that if these variables could be investigated under laboratory conditions, it would be very helpful in arriving at information on which to base more realistic controls.

Dr. Prakash noted that Mr. McFarren's paper contained a slight error and pointed out that toxicity levels on the Canadian Atlantic coast have not successively decreased since 1957.

Dr. Loosanoff also indicated that he had received a report from Dr. Korringa of cases of paralytic shellfish poisoning in Holland in 1961.

Dr. Tennant inquired of Mr. McFarren if he knew of anyone who has worked on the limits of error in the mouse test, and sought his opinion as to the number of animals that should be used in a laboratory test. Mr. McFarren replied that the test is probably accurate within plus or minus 20 percent and, under ideal conditions, might be accurate to plus or minus

10 percent. On the basis of collaborative studies using the CF value, it is his belief that three animals are sufficient for a definitive test provided the death times are in the five to seven minute range. Dr. Tennant replied that his experience raised a question as to the adequacy of three animals per test, and stated there is tendency among laboratory workers to expect a higher degree of accuracy from the test than is actually obtainable.

Mr. Robert Brown, Chairman, thanked the many participants in the Workshop and declared the 1961 Workshop to be adjourned.

APPENDIX A

statement by

Dr. Robert J. Anderson, Chief
Bureau of State Services
Public Health Service

For the Surgeon General, for the Public Health Service, for all of us who are interested in this subject of shellfish sanitation, I welcome you to this Fourth Conference on shellfish sanitation. We are heartened by the fact that there are about 120 persons who have registered for this meeting. As Mr. Woodfield said, this is probably the largest session of this sort that has taken place. It indicates the interest that all of us have in an important source of food supply for this country and for other countries of the world and a delectable source of food supply for people too.

We really have one common interest, and that is to make this food supply available to our people in such a fashion that they benefit from the fine properties of the food and that they do not incur any unnecessary risks in the process of enjoying the benefits of the food.

We welcome also the Canadian members of this conference who, along with us in the United States, have been participants in these affairs since the inception of these conferences in 1925.

We also welcome our observers from Japan who have come so far to be with us in a joint endeavor. This meeting can be one of the most important meetings held on this subject since the start of these meetings.

Going back in history, as I understand it, one of the things that led to this sanitation workshop in its outset was a problem of disease transmission through the media of shellfish. Typhoid, I believe, in 1925, pointed out certain problems that were present in this food chain. Here within the past year we have again seen the importance of the endeavors of the industry, the endeavors of State agencies, the endeavors of the Public Health Service to preserve the quality of the food supply. I do not need to relate to you the specifics of the two outbreaks of hepatitis occurring as a consequence of breakdowns in procedures in Pascagoula, Mississippi, and in the Raritan Bay, New York and New Jersey. I do not want to dwell on these events.

Rather, I do think that it is important that all of us look at these events from the standpoint of the program defects or the vulnerable spots for all of us that these two events portrayed. These defects must be studied by all of us from the standpoint of remedying them or else we are going to be in continuing trouble.

The problems that were pointed out were of two sorts. One type of problem was the technical kind of problem relating to what happens to viruses in waters. How long do they survive? How do they escape or get into growing waters? How do they pass through sewage treatment plants? What happens to these viruses within shellfish? Are they destroyed as are bacteria rapidly, or do they reside for longer periods of time in shellfish than do bacteria?

Another technical problem is: Once oysters do acquire viruses, how long do they survive and how can they be removed? A second kind of problem, however, is the administrative kind of a problem with regard to practical operation.

What were the deficiencies and the defects in programs which stand out to attract our attention? I think all of us appreciate that under the law, under the national law, the Public Health Service has responsibility for the control of the interstate movement of disease. Within the States, there is a responsibility within that State for the protection of the health of the citizens of that State.

In the development of the cooperative program, the cooperative certification program, however, the States have accepted responsibility for assuring the sanitary control of shellfish production and the whole program rests on how well this responsibility that has been assumed by the States is performed. Our responsibilities in the Public Health Service under this certification program are to assure ourselves that the State program is operating satisfactorily and that we can endorse those State programs which are acceptable.

This means that we must review with you the operation of programs within the States. We hope that you will be in a position that you will be so knowledgeable about your program that you will be able to document the administrative aspects of the program in such a fashion that there is clear proof that your program merits endorsement. We think that if you know that you have defects in your State and you do not point these defects out within your State to us and to the industry that then the whole program, the whole industry is in jeopardy. The whole cooperative State-Federal endeavor and industry endeavor rests upon the fact that we must operate with full, complete interchange of information and knowledge about what the true situation of our control efforts is.

For myself and for the Public Health Service, we feel we cannot continue to be a party to a voluntary certification program if the States and the industry will not function on a free and easy basis. That is why we regard conferences of this type as being so important because here is where we lay the problems out on the table, dissect them and work out corrective measures.

You know that our regional office staff are the people upon whom we depend to work with industry and with the States in developing the kinds of assurances that we all need about the sanitary quality of shellfish.

Now, one of the administrative problems that I am sure all of us have had has been that we have not had funds, money, in some instances to carry out the responsibilities that we know technically we should be carrying out to assure safe shellfish. We do ourselves a disservice. We do other States and the industry a disservice if we do not point out these lacks and needs and the import of these lacks and needs as it relates to the certification program.

To my way of thinking, I do not want to have any kind of a feeling that we are putting interstate certification tags on shipments of shellfish moving from one State to another and, in effect, are putting the Public Health Service's stamp of approval on those shellfish if I have a feeling that the program people in our regional offices and in the industry and in the States know that they are operating dangerously. To do this jeopardizes all of us.

So we need, I think, a realistic appraisal here of the financial and technical needs of State programs. Let us not kid ourselves. One or two people cannot do a job that might require ten or twenty people. On the other hand, a real question comes up, I think, with regard to the economics of this program as to whether in all instances the extension of the program to its fullest extent might be justified in terms of the economics of the oyster or the shellfish industry within that State. This is a question that, of course, is going to be a hard one to tangle with. It probably best can be coped with within the State.

However, if you have all areas of a State open or under a shellfish certification program, we must, in fact, have the people to make certain that the areas can be open.

This past year, I think, also is significant in another regard from the standpoint of the national program in that the last session of Congress did appropriate monies for the establishment of two shellfish sanitation research laboratories. One of these will be located at Kingston, Rhode Island, where the University of Rhode Island has a very good marine biological laboratory, where there is also going to be located one of the ten oceanographic laboratories under the National Oceanographic Program.

The other laboratory is going to be located on the Gulf Coast at Dauphin Island, which is at the mouth of Mobile Bay as it goes into the Gulf where another important segment of the industry operates. The programs of these laboratories, like that of the laboratory that we have on the West Coast at Purdy, Washington, will be in part research and the research that will be undergoing at these sites will be research with regard to the passage of toxic chemicals and physical agents, radioactive substances, for example into and out of shellfish and methods which might be used to abort or avert the effects of these substances upon the shellfish food chain.

We also hope to have at these laboratories sufficient staff so that our regional offices will have available additional technical consultation for the States to take up special problems. We are also planning to increase the number of people that we have in some of our regional offices to work with the States in developing better State programs. We want these centers to be of assistance on the problems that we have in this field. We hope that these centers will be fully operational within the coming year.

Now, one other subject impresses me, and that is the fact that this program has been operating on a voluntary basis as far as the Federal-State relationship is concerned. I think this is fine. But we cannot forget that the program does also operate within the framework of the National Health Service's responsibility to control the interstate spread of disease.

In the past two sessions of Congress, legislation has been introduced to specifically identify the national agencies' responsibilities with regard to certain interstate aspects of this program. The question comes up as to whether this legislation might not be extended to provide specifically for all phases rather than just certain aspects of the interstate program. Specific aspects which the legislation has approached have been that of the import problem, the problem regarding the sanitary control of imported shellfish. But when you stop to think that, I believe, there is something like 18 States themselves that do not have any control measures, any legislative measures within their States regarding the sanitary quality of shellfish, I think you will get a picture as to the need for a national uniform approach to this kind of a problem. It is my personal belief that it is badly needed from the standpoint of the industry, from the standpoint of the Public Health Service and from the standpoint of the States.

We hope here that this conference will be, as Dave said, a free and easy one with all of you raising questions, making comments which are pertinent to the subjects under discussion. In that framework, I am sure the conference will be productive and that the industry and the official agencies that are represented here will begin to work out some of the solutions which are needed to the many administrative and technical problems that face us.

I am not so sanguine as to hope that you will work out all of the problems here today, but the important thing is that we get started on working them out and that we develop a philosophy which we all understand in our approach to these problems. I wish you luck in your endeavors.

APPENDIX B

THE APPLICATION OF THE "CONDITIONALLY APPROVED" SHELLFISH GROWING AREA CONCEPT

by

Eugene T. Jensen
Shellfish Sanitation Branch

The Conditionally Approved area concept has for a number of years been the most controversial and perhaps the least understood element of the Cooperative Program for the Certification of Interstate Shellfish Shippers. A number of States deny the existence of problems which can be met only by the application of this concept. It is perhaps the most important single element of our shellfish sanitation program, despite the fact that the term has not been adopted by most States. In fact, the future of both the shellfish industry, as we now know it, and the Cooperative Program may well depend on the proper application of this concept.

The purpose of this presentation is to discuss the technical elements underlying the conditionally approved area concept, to evaluate the application of the concept by the States and to explore its possibilities for the future. There is an urgent need for such discussion! The recent experiences in Mississippi, New Jersey and Connecticut associating infectious hepatitis with the consumption of raw shellfish demonstrates forcefully that the States must make critical evaluations of the sanitary quality of those shellfish growing areas which receive effluents from sewage treatment plants. (1, 2, 3) The State shellfish sanitation control agencies must understand the technical limitations of sewage treatment. They must also understand how these limitations influence the planning of growing area surveys and particularly the interpretation of the results of bacteriological sampling of the growing area waters.

TECHNICAL BASIS FOR SHELLFISH SANITATION PROGRAM

An understanding of the conditionally approved area concept is dependent upon an understanding of the original premises upon which the Cooperative Program was founded, and upon recognition of the technical advances which have been registered since that time. As is generally known, a public-health interest in shellfish sanitation developed in this country early in the 1900's. (4) The relationship between disease transmission by shellfish and sewage pollution of growing areas or storage areas was identified by these early public-health workers. Consequently, the State and the Federal agencies took many steps to evaluate the sanitary quality of growing areas and to develop a control system which would be effective in preventing the spread of communicable diseases due to shellfish taken from polluted sources. Although considerable effort was expended, a coordinated intergovernmental approach to shellfish sanitation was not developed until 1925. At that time a major outbreak of typhoid fever occurred attributed to polluted oysters which led the States, the Federal agencies, and the shellfish industry to adopt a coordinated, uniform system for the sanitary control of the shellfish industry. This was the beginning of the Cooperative Program for the Certification of Interstate Shellfish Shippers. It is a story with which many public-health workers are familiar. (5)

The sanitary survey of shellfish growing areas was perhaps the most important single component of this early certification program. It is interesting to note that the 1925 report of the founding committee suggested "Areas must be so protected against existing or probable sources of pollution by artificial treatment of sewage when it is necessary and by the natural safeguards of dilution of time." Relatively few coastal cities had sewage treatment in the 1920's. Early workers in shellfish sanitation were ordinarily concerned with the sanitary evaluation of estuaries which received relatively constant amounts of pollution. Under these constant conditions, a study could readily be made of pollution sources, currents, flow time, and bacteriological quality of the water which would essentially define that portion of the area which was contaminated. Such a situation is illustrated in Figure 1.

In 1925 the relationship between sewage treatment plants and shellfish growing areas was therefore not an item of major concern to either the shellfish sanitation agencies or the shellfish industry. Vast areas of the United States coastal waters were essentially free of pollution and available to the shellfish industry. In addition, the national population was then relatively small, and except in the vicinity of a few major cities, the effects of pollution were not widespread. Consequently, the closure of small marine areas in the immediate vicinity of sewer outfalls did not have severe economic consequences for the shellfish industry.

In the period following World War II, several developments combined to present shellfish sanitation control authorities and the shellfish industry with a series of new and difficult problems. The most fundamental change was the urbanization of many previously sparsely populated coastal regions. The development of new highway systems, the increased mobility of our population, and the large amount of time available for recreation resulted in major population changes in many previously lightly populated coastal areas. In many instances this increase in population was paralleled by industrial developments. The obvious advantages of coastal locations--transportation and water supply--drew many of these industries into the same coastal areas.

The history of water pollution control measures in the United States is well documented and need not be discussed in detail at this point. The Herculean efforts of citizens groups, conservationists and many others to develop a national water pollution control program to assure the safety and adequacy of the nation's future water supply is a matter of public record. Eventually this force resulted in the adoption of a federal water pollution control act, and a system where the Federal government could provide at least limited financial assistance to communities for the construction of pollution control works.

This construction program has had an impact on both inland and coastal areas. Increasing numbers of our coastal communities have installed interceptor sewers and sewage treatment plants. Unfortunately, there has not always been good coordination between the agencies responsible for the promotion and design of sewage treatment facilities and the agencies usually municipal, responsible for their operation. One might justifiably ask "of what concern is this to the shellfish sanitarian?" It is a matter of real concern. In fact, from the standpoint of the shellfish sanitarian, there is an essential need for effective three-way coordination between the sewage treatment plant, the designer, the operating agencies, and the agencies responsible for the safety of the shellfish. More will be said on this point.

A clean distinction has not always been made between theoretical and practical benefits which can accrue from adequate waste treatment. In the former, the sewage treatment plant is viewed as an absolute device which is capable of removing a predetermined proportion of the offensive material. In the mind of the citizen, the sewage treatment system is apparently thought of as the complete and absolute removal of all offensive material. From a more practical standpoint, a sewerage system is frequently a complex machine which can ordinarily accomplish its design objectives, but with a realization that many mechanical and human factors will interfere with its operation. Consequently, the degree of sewage treatment (in this case the percentage of bacteria which might be removed) will vary greatly from hour to hour and from day to day. The original design limitations are also an important consideration. Few sewage treatment systems result in the elimination of all, or essentially all, of the microorganisms. In many systems a 50 to 90 percent reduction is a more realistic value. Thus a city of 10,000 people with a sewage treatment effectiveness of 90 percent is the equivalent of a city of 1,000 with no sewage treatment.

The reliability of sewage treatment is greatly influenced by the type of treatment provided and by the size of the community which is involved. Some forms of sewage treatment involve little mechanical equipment and require a low degree of skill for operation. Other methods of sewage treatment (including the so-called "packaged" sewage treatment plants) have considerable mechanical equipment and require a relatively high degree of skill for proper operation and maintenance. Many small communities necessarily delegate the responsibility for sewage treatment plant operation to a single individual who may also be responsible for maintenance of the water system, street repairs, and similar duties. In such communities the treatment plant is ordinarily unattended during the evening hours, weekends, holidays, and periods in which the operator, for many other reasons, may not be able to work. Similarly, long delays may be anticipated in the repair of equipment. In contrast, a large municipality or sewage district may have a more adequate staff which can provide essentially around-the-clock, 7 day a week maintenance and operation.

The political science aspects of sewage treatment must also be considered and its impact recognized by those responsible for the sanitary production of shellfish. When a community has invested large sums in the construction of a pollution abatement system they are justified in their belief that tangible benefits should accrue therefrom. When the protection of shellfish growing areas has been used as a partial justification for the construction of these facilities, the community may be expected to assume that the level of pollution will be changed with the completion of the sewage treatment plant, and that additional nearby marine areas can be made available for shellfish harvesting. The shellfish industry in the area may have similar high hopes. In fact, the shellfish industry may have been instrumental in convincing the municipality of its obligation for providing effective treatment of its wastes.

THE DEVELOPMENT OF THE CONDITIONALLY APPROVED AREA CONCEPT

In the early 1950's the Public Health Service recognized the trend towards the construction of many additional sewage treatment plants in the coastal areas and the probable technical impact on shellfish sanitation procedures. It was apparent that the implementation of federal construction grants would accelerate this trend and that the construction of sewage treatment plants would completely change the premise upon which the shellfish sanitation program had been founded, i.e., that the level of pollution in an estuary may be readily defined by a combined sanitary engineering-bacteriological survey.

The criteria for the classification of shellfish growing areas were based on the long-established principles that the quantity of sewage and point of discharge would remain essentially constant; that the sewage would be uniformly mixed with clean diluting water; and that the mixture of sewage and dilution water would be distributed uniformly over the shellfish area. When a sewage treatment plant is constructed in a coastal community, this entire relationship is destroyed. A hypothetical situation is shown in Figure 2. In this example, the city in Figure 1 has constructed a sewage treatment plant. If the plant is operating properly the area previously designated as "Restricted" should ordinarily have a very low level of pollution. It may be expected that both the municipality and the local shellfish industry will bring pressure to bear upon the shellfish control agencies to make this area available for harvesting as a fully Approved area. These groups will cite the fact that the pollution problem has been solved by the construction of a sewage treatment plant and may bring major pressures to bear to have a bacteriological survey made to prove that the area is no longer polluted. In some instances, the municipality or the industry may even collect samples of water or shellfish to "prove" that the area is safe for the harvesting of shellfish for direct marketing.

Are such actions justified? There is no one answer to such a complex question. However, there is a sequence of related natural laws which the shellfish sanitarian must recognize and evaluate:

(1) Contrary to public opinion, a sewage treatment plant does not ordinarily remove all bacteria from the sewage.

(2) Chlorination, which is responsible for much of the decrease in bacteria, is especially prone to failure because of the corrosive nature of chlorine and the resulting need for careful maintenance of equipment. The effect of sewage treatment on viruses is problematical although it has been determined that enteric viruses are less numerous in treated than in raw sewage. (6)

(3) Treatment plants, particularly those of smaller municipalities, may be unattended at night, on weekends and holidays.

(4) The effectiveness of a sewage treatment plant may vary with the nature and quantity of sewage which must be treated.

(5) The point of discharge of sewage may vary. (Such a situation is illustrated in Figure 3. In such a situation any overflow from the pumping station at point A would effect the sanitary quality of the growing area. Overflows might be caused by poor maintenance, excessive infiltration, storm flow from combined sewers or unavoidable power failure.)

(6) Shellfish will reflect rapidly any deterioration in the bacteriological quality of their environment, but are slower to reflect improvement.

From a consideration of these factors, one is led to an inescapable conclusion that an estuary which receives effluent from a sewage treatment plant must be considered as a special case. Any variation in sewage treatment will effect the sanitary quality of the water in the estuary. In turn, the shellfish will rapidly reflect any downgrading of the aquatic environment.

In recognition of the variable sanitary nature of estuaries that receive effluent from sewage treatment plants, the Public Health Service in 1956 proposed that consideration be given to the establishment of a new category of shellfish growing areas--"Conditionally Approved." The term "conditionally approved" is in itself of little consequence. The concept is fundamental! Simply stated, this concept holds that an area of variable sanitary quality shall be approved for shellfish harvesting only under certain prescribed "conditions." If these conditions are not met, the area must automatically revert to a prohibited or restricted status. The concept is not new. Similar thinking has been applied for years in other closely related technical areas such as marine toxin. For example, areas are approved for harvesting only during those periods in which shellfish are essentially free of paralytic shellfish poison. (7) If this "condition" is not met, i.e., the shellfish show evidence of accumulating poison, the area will be closed to harvesting. As proposed by the Public Health Service in the 1956 Shellfish Sanitation Workshop, the application of the conditionally approved concept was not limited to areas which receive effluent from sewage treatment plants. It was intended to apply equally to areas in which the sanitary conditions might depend upon other factors such as stream flow, summer population, or use of marinas or ship anchorages.

These proposals for a change in the basic sanitary requirement of the Cooperative Program were circulated widely to the State agencies, the other Federal agencies and the shellfish industry. These proposals were discussed in detail at the 1958 Shellfish Sanitation Workshop. (8) While many States objected to the use of the term "conditionally approved", there was no serious objection to the adoption of the philosophy. Part I of the shellfish sanitation manual, (9) adopted at the 1958 Workshop, therefore provides that the States may designate areas as conditionally approved under certain specified conditions.

USE OF THE CONDITIONALLY APPROVED AREA

The conditionally approved area concept has now been used as an integral part of the Cooperative Program for three years. In view of its importance in the production of shellfish of acceptable sanitary quality, the Public Health Service has attempted to evaluate the degree to which the concept has been used by the States, the degree to which it is understood by industry, design engineers, and communities, and to try to determine the future course of action which shellfish sanitarians should take with respect to the use of this concept. Three general approaches were used in making this evaluation:

(1) A review of operating experience in those areas where the conditionally approved concept was used.

(2) An appraisal of State sanitary surveys to determine the extent to which sewage treatment factors had been considered in area classification.

(3) Visits to sewage treatment plants in many areas of the country to determine the attitude of operators, and to attempt to ascertain the degree of coordination between the pollution control, treatment plant operating, and shellfish sanitation authorities.*

Due to a number of uncontrolable factors, it was not possible to carry out the survey with the throughness that had been intended. However, it is believed that the information obtained is representative of the national problem, and is generally satisfactory for use in the administration of the Cooperative Program.

The results of our studies are best described as depressing. While there are a few bright spots, it appears that few agencies have taken adequate steps to acquaint themselves with the problems involved in the classification of areas which receive effluents from sewage treatment plants.

Successful Applications:

One State has applied the concept successfully. Previous experience has shown that the sanitary quality of one of the State's major shellfish production areas depended upon the effective operation of a 14 MGD sewage treatment plant and two large pumping stations. The sewerage system is operated by a well organized, well-staffed Sanitary District. A fail-safe alarm system has been installed in the pumping stations and sewage treatment plant so that any malfunctioning is detected immediately. The detection-notification-reclassification procedure for this area can be performed in less than two hours. The area is reclassified several times a year which requires a great deal of administrative work. However, the application of the conditonally approved concept has permitted 2.5 million dollars worth of shellfish to be harvested annually from the area.

In another State a major shellfish production center was established following the construction of municipal sewage treatment facilities. Studies made by the State indicate that a short term failure of the treatment devices would not result in pollution of the areas which have been approved for shellfish harvesting.

It must also be noted that a number of the States have used a system whereby areas are closed to shellfish harvesting during periods of maximum summer population or during periods of high stream flow with an attendant increase in pollution levels of the estuaries. A system of this type has also been adopted by the Japanese in connection with the discharge of the River Ohta into Hiroshima Bay.

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* These visits were made in connection with the routine Public Health Service's evaluation of State shellfish sanitation programs.

Evaluations of sanitary surveys:

Our review of the adequacy of sanitary surveys made by the States was discouraging. It was found that about two-thirds of the surveys examined were not sufficient to determine whether the quality of the Approved areas was or was not dependent on the operation of a sewage treatment plant. In a few instances, Approved area boundaries had been delineated by "rule of thumb," i.e., prohibiting shellfish harvesting within a one-half mile radius of the sewage outfall withour respect to size. In many cases it appeared that the classifications were based largely on the results of bacteriological examination of water. Almost without exception, the bacteriological results had not been analyzed in terms of the performance of the sewage treatment plants, i.e., the degree of sewage treatment at the time of sample collection was not known.

An example of the difficulties in evaluating the results of bacteriological sampling is shown in the attached probability plot. (Figure 4) The median MPN obtained from the series of 16 samples is 49. However, upon statistical analysis shows that an unusual distribution existed! In fact it appears that two distinct conditions prevailed--during one period, the area was almost free of pollution while at other times the bacteriological data was suggestive of a relatively high level of pollution

In general, it appears that States have continued to use 1925 sanitary survey procedures which were based on the premise that there was both a constant source and quantity of pollution.

Operational problems:

Discussions with the State agencies responsible for shellfish sanitation suggest that there is poor coordination between the water pollution control groups responsible for approval of growing areas. Understandably, the agencies responsible for sewage treatment plant construction and operation do not wish to have these facilities under routine inspection of a second State agency. However, unless a system can be developed whereby sanitary survey data for shellfish growing areas can be interpreted in terms of an hour by hour, day to day evaluation of sewage treatment results, the State shellfish sanitation control agencies will have no choice but to prevent harvesting for direct marketing from areas of variable sanitary quality. By the same line of reasoning, the Public Health Service cannot endorse the adequacy of a State shellfish sanitation program when it is in the possession of knowledge that adequate surveys have not been made of shellfish growing areas or where provisions have not been made to assure the prompt closure of variable quality areas in the event sewage treatment plant failure or malfunctioning.

It must be emphasized that the administrative problems involved in the approval of shellfish harvesting from areas of highly variable sanitary quality are exceedingly complex. In many instances the construction of sewage treatment facilities by a small municipality will not allow the opening of additional shellfish harvesting areas, although it may alleviate local "pollution" conditions. This is, of course, a generalization and where a municipality does have a reliable sewage treatment plant and where the distance separating the sewage treatment plant from the shellfish growing areas is such as to allow for the functioning of the administrative system, it should then be possible to designate the area as conditionally approved and to permit the harvesting of shellfish under specified conditions.

A reasonably complete review has been made of all potential conditionally approved growing areas in one PHS region, i.e., areas which are/or can be affected by sewage treatment.

It is almost impossible to summarize the data collected in such an evaluation. However, a few of the more significant statistics may help to define the magnitude of this problem.

Number of areas influenced:*	24
Number of treatment plants	58
Average distance of shellfish growing areas to sewage treatment plants	6.4 miles
Total flow of sewage MGD	105
Size and range of sewage treatment plants - MGD	0.01 - 36.0

Clearly, the variable quality area is a major consideration in many States. We also find it interesting to note that in one or two instances a State is considering a conditionally-approved agreement where the sewage treatment plant is only 0.5 to 2.0 miles from shellfish growing areas.

Sewage Treatment Plant Operations:

The attitude of operators, especially in small plants, is also a consideration. The following three examples of conversations of our filed staff men with sewage treatment plant operators suggest the type of problem involved.

Case 1:

A primary sewage treatment plant was discharging 10,000,000 gallons of sewage per day at a distance of 2 miles from an Approved shellfish growing area. While visiting the plant, it was found that the chlorinator had been inoperative for 48 hours. The operator was asked if he had contacted the State Department of Health relative to this breakdown. His reply: 'Why should I contact them--they can't help me fix it!"

Case 2:

A primary sewage treatment plant was discharging 2,000,000 gallons of sewage per day at a distance of one-half mile from an Approved shellfish growing area. The operator indicated that he planned to by-pass raw sewage for two days while repairing the sedimentation tanks. When asked who he planned to notify of the proposed by-pass, he answered, "I usually notify the Borough Hall Clerk."

Case 3:

During the conversation with the operator of a sewage treatment plant, it was discovered that the pumping station located at a distance of one-half mile form an Approved shellfish growing area had been by-passing sewage for 10 hours at a rate of 400,000 gallons per day. The State Department of Health had not been notified of the situation.

There are numerous other examples in our files. Insofar as shellfish sanitation is concerned, these experiences lead to the inescapable conclusion that effective sewage treatment operations is particularly lacking in the smaller communities. Perhaps this need not necessarily be so, but at the present time it does seem to be a well established fact!

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* Not all areas currently have commercial quantities of shellfish. However, it must be realized that shellfish are a biological resource and that given the proper condition marketable quantities might be developed. The areas in question have all been classified.

VIRUSES AND SEWAGE TREATMENT

The recent association of the transmission of infectious hepatitis by shellfish which are eaten raw, further complicates the appraisal of the conditionally approved area concept. The safety margin inherent in our classification criteria has never been really established. Some agencies have previously suggested that it is very large, and that we could safely ignore the refinement offered by the conditionally approved area concept. Their position was based on the fact that no outbreaks of communicable diseases had been attributed to shellfish over a substantial period of time, although small quantities of shellfish undoubtedly had been harvested surreptitiously from areas which were not fully approved. This contention may or may not have been ture. The fact remains that the transmission of pathogenic viruses by shellfish poses a host of technical complications. On the basis of studies which have been made, we feel reasonably confident that the classification criteria for Approved areas afford a reasonable safety margin and that shellfish harvested from areas meeting this criteria will not be involved in transmission of infectious hepatitis. We also know that sewage treatment processes do reduce the number of virus particles, although this knowledge is to some extent, countered by that which indicates that virus inactiviation by chlorine is hindered by the presence of large quantities of organic matter (6, 10) The fact that chlorination may destroy our indicator organism, coliforms or E. coli, without having an equivalent effect on the viruses further complicates the picture.

THE FUTURE OF THE CONDITIONALLY APPROVED AREA

Nationally, the pace of construction of sewage treatment plants continues to increase. As officials responsible for the sanitary production of shellfish we will be faced with the administrative control of increasing numbers of areas which are of variably sanitary quality and which therefore, can be considered for classification as conditionally approved.

The conditionally approved area concept has been developed to meet a specific need. Studies which have been made by the Public Health Service suggests that many areas now classified by States as "Approved" should, in fact, be classified as "Conditionally Approved." Experience also suggested that the conditionally approved area concept cannot be applied safely in many areas, particularly those in which a small municipality is responsible for the sewage treatment plant operation.* We believe very strongly that the various States must recognize and accept the relationship between sewage treatment and the sanitary qualities of the shellfish growing areas, and that action must be taken to classify areas on the basis of the criteria agreed-upon in the Cooperative Program.

It is not incumbent upon the States to use the conditionally approved classification in name or in fact. However, if an area is approved without consideration of the effect of sewage treatment effluent, the Public Health Service will have no choice other than to reflect this deficiency in program evaluations, and if serious hazards exist, to terminate endorsement of the State's program.

As an alternate to the use of the conditionally approved concept, the Cooperative Program could adopt a position whereby areas would be classified as if no sewage treatment had been provided. This would be the most conservative approach; however, in many instances State agencies might find it politically hazardous to adopt or try to defend such an extreme and vulnerable position. More extensive use of relaying shellfish from polluted to clean areas is an alternative to the use of either the conditionally approved area, or an absolute restriction on the use of shellfish resources in areas which are occasionally subject to pollution. Relaying has been practiced for many years and is a proven method for providing protection against the transmission of bacterial diseases by shellfish. It is not necessarily effective with the marine poisons or with industrial wastes. Similarly, there is no real evidence which shows that the process is either effective or ineffective in the removal of viruses; however, there is no epidemiologic evidence linking relayed shellfish and the transmission of viral diseases.

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* Obviously, the factors of distance, quantities of sewage and type of treatment are important considerations.

Many shellfish sanitarians have long maintained that artificial purification affords the best method of controlling the sanitary quality of shellfish. This position is based on the supposition that methods can be developed which are both economically and technically practical. The method has been used with a high degree of success in several foreign nations; however, it has had only limited acceptance in the United States. Again, we must recognize that artificial purification procedures, while effective with bacteria, may not have an equivalent effect on marine toxins, industrial chemicals, and viruses. Certainly it will be necessary that these factors be investigated before the Cooperative Program can seriously recommend the controlled purification of all shellfish marketed in the United States.

Some analyses of the cost of these various alternatives should perhaps be made. Extremely expensive sewage treatment methods may be required where the protection of a shellfish growing area is a primary objective. In some instances, it may be more practical to construct a less expensive sewerage system which would meet other pollution control objectives but which would not necessarily provide the high order of safety necessary for shellfish harvesting. In this situation it might be economically appealing to the community to underwrite all or a portion of the cost of a shellfish relaying or purification operation in preference to the construction and maintenance of the more expensive sewage treatment process.

SPECIFIC PUBLIC HEALTH SERVICE RECOMMENDATIONS

In consideration of those factors governing the harvesting of shellfish from areas which are subject to occasional pollution, it is suggested that the following actions be considered by the Workshop:

(1) Continued use of Conditionally Approved Classification:

It is recommended that the conditionally approved classification be retained in the Cooperative Program for the Certification of Interstate Shellfish Shippers. In view of the recent transmission of viruses by shellfish it is further recommended that States adopt a conservative attitude with respect to the use of this classification, and the conditionally approved areas not be established unless all satisfactory compliance items of Section C-4, Part I of the shellfish manual are met. Emphasis should be placed on the development of an effective system for assuring the closure of shellfish harvesting areas whenever there is a decrease in the level of sewage treatment. It is equally essential that a conservative attitude be observed in the establishment of a closed safety zone of adequate size between the conditionally approved area and the source of pollution. In most instances, a minimum flow time of two days should be allowed to assure that the agency will have a reasonable opportunity to be informed of a failure of sewage treatment and to take the necessary action to close the growing areas. In general, it is suggested that conditionally approved areas not be established in situations in which the municipality is unable to provide full-time operators for the sewage treatment plants unless the factors of distance, quantity, and type of treatment are such that the shellfish sanitation control agency can be assured that the area will, in fact, be protected against sewage pollution.

(2) Sanitary Surveys of Growing Areas:

It is recommended that the States give adequate considerations to the effect of sewage plant effluents when making sanitary surveys, and that bacteriological sampling be interpreted in terms of the level of treatment known to prevail at the time of sample collection.

(3) Interagency Agreements:

It is recommended that effective operating relationships be established between the State agencies responsible for water pollution control activities and those responsible for the sanitary quality of marine food resources. The fact that a sewage treatment plant has been constructed cannot be accepted as prima facie evidence that the area has been freed of pollution. Written interagency agreements outlining objectives and areas of responsibility should be developed wherever possible. Formal agreements are also suggested in the case of the conditionally approved areas to insure that all interested parties, including industry, are aware of the technical limitations.

(4) Relaying:

It is recommended that relaying be accepted as a matter of policy as affording a larger safety margin than that offered by the conditionally approved area. In view of the lack of knowledge regarding the ability of shellfish to eliminate viruses, it is urged that shellfish be held in the relaying area for an extended period of time. The period of time involved would seemingly depend upon temperature and other factors influencing the physiological activity of the animals. It might be noted that the previous edition of the shellfish manual contained a requirement that shellfish remain in the relaying area for a period of at least fourteen consecutive days, during which period the temperature of the water was to be constantly above 50° F. (11)

(5) Artificial Purification:

It is recommended that investigation of purification procedures be encouraged particularly with respect to the ability of shellfish to eliminate viruses. Public Health Service long-range research plans emphasize the continuing investigation of purification processes and the possible development of practical methods for large scale purification of shellfish. It is anticipated that the New England shellfish sanitation research center will include a pilot plant for such studies.

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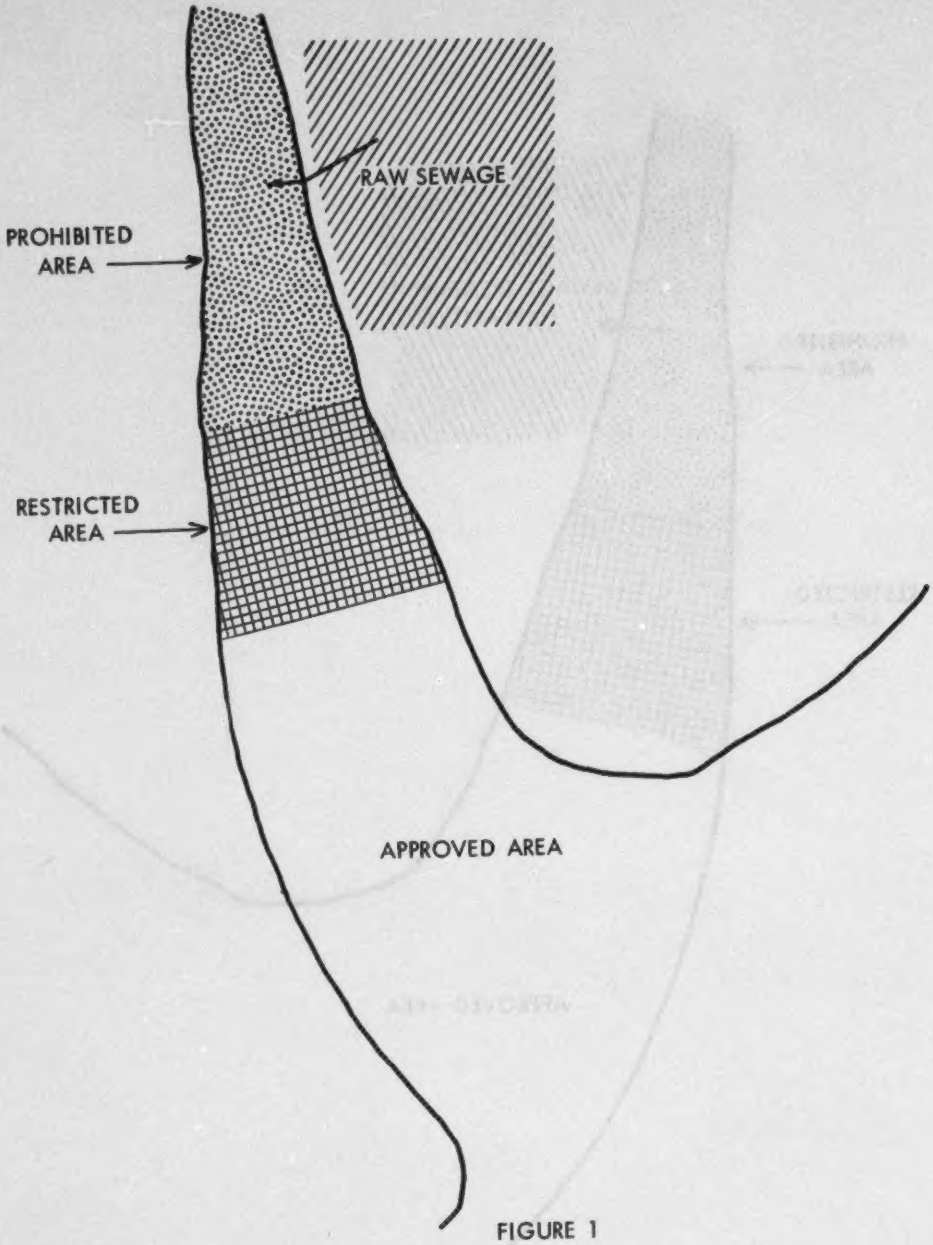

RAW SEWAGE
PROHIBITED AREA
RESTRICTED AREA
APPROVED AREA

FIGURE 1

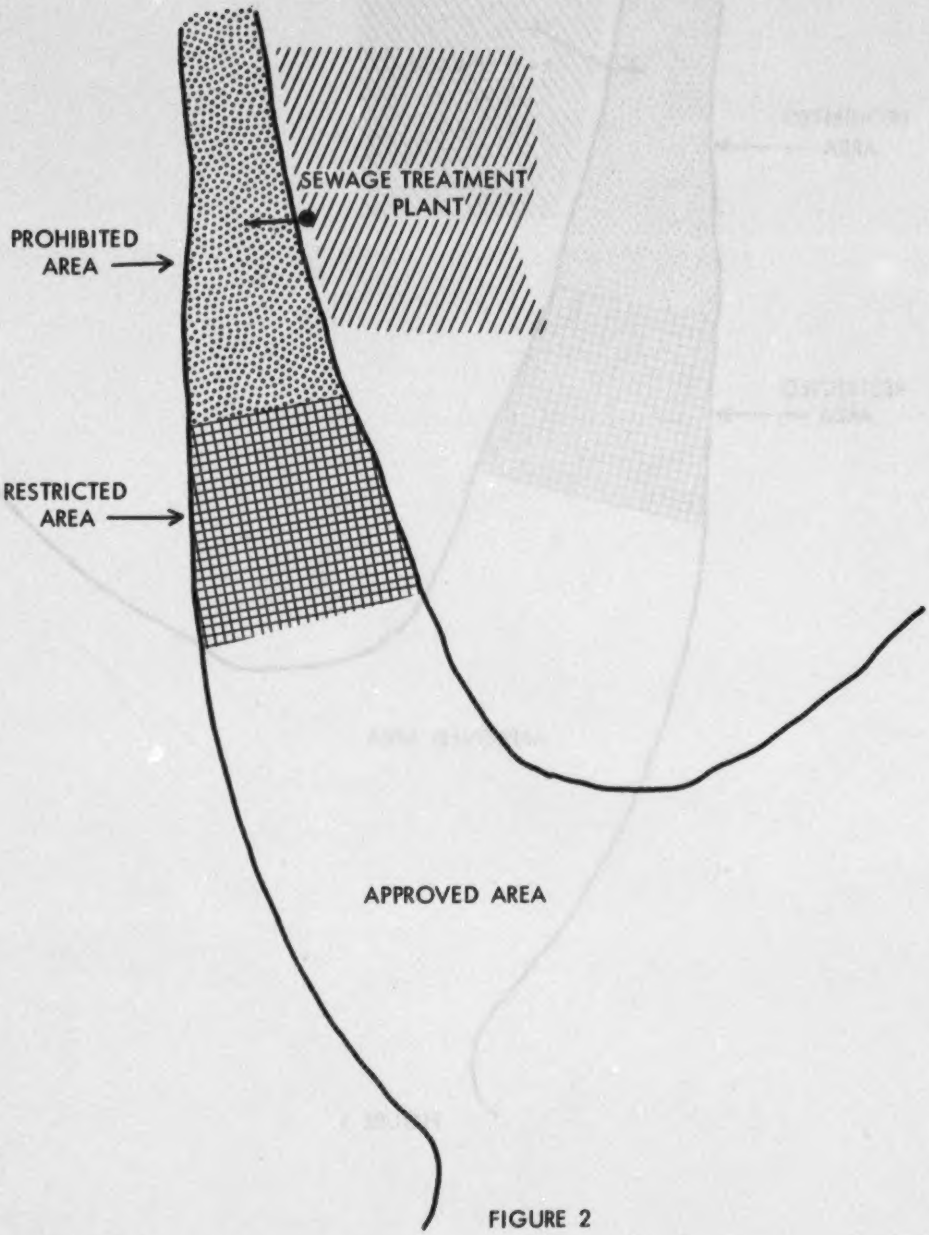
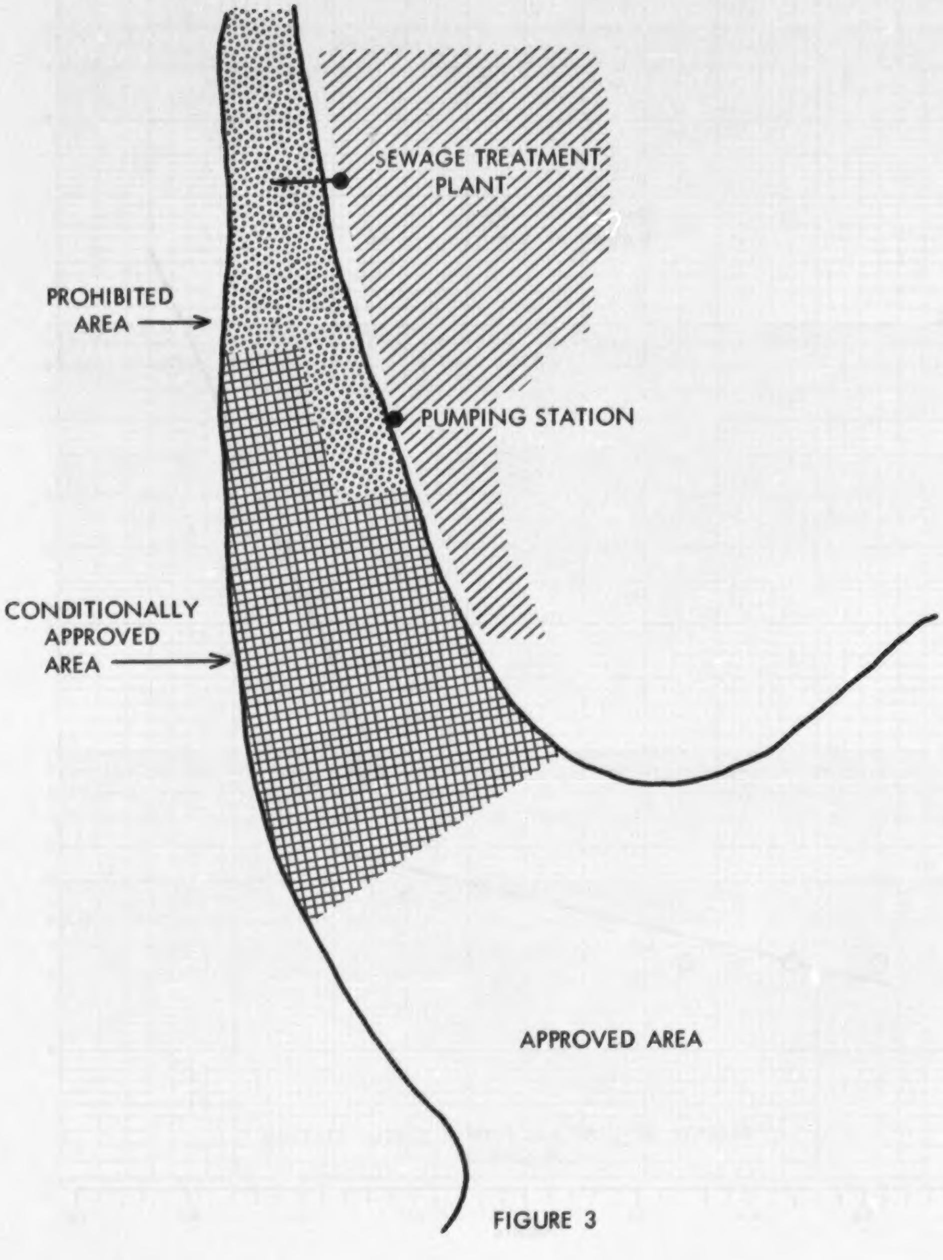
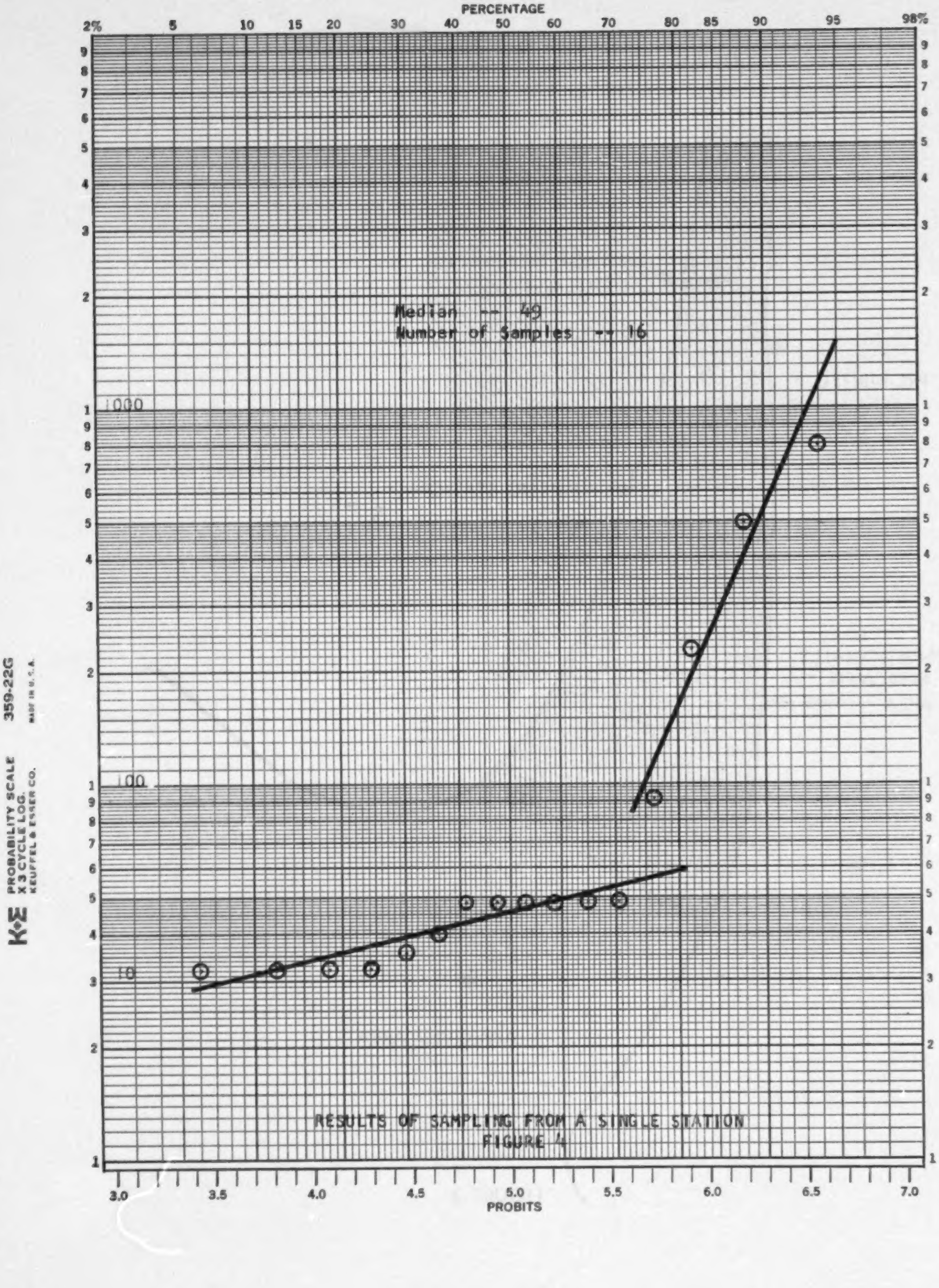

SEWAGE TREATMENT PLANT
PROHIBITED AREA
RESTRICTED AREA
APPROVED AREA

FIGURE 2



SEWAGE TREATMENT PLANT
PROHIBITED AREA
PUMPING STATION
CONDITIONALLY APPROVED AREA
APPROVED AREA

FIGURE 3



Median -- 49
Number of Samples -- 16

RESULTS OF SAMPLING FROM A SINGLE STATION
FIGURE 4

APPENDIX C

PUBLIC HEALTH SERVICE POSITION WITH RESPECT TO THE SANITARY CONTROL OF SHELLFISH IMPORTS

by

John D. Faulkner
and
Eugene T. Jensen
Public Health Service

The sanitary control of shellfish shipped in international commerce has for some time been a matter of concern to the shellfish industry and to officials responsible for the administration of the Cooperative Program for the Certification of Interstate Shellfish Shippers. However, this is not an entirely new problem. When the program was organized in 1925, Canada, then an importer of oysters from the United States, requested that we adopt a system whereby only oysters of acceptable sanitary quality would be exported to Canada. To facilitate this control of exports, the system of numbered certificates for shippers was embodied in the Cooperative Program. In 1948 this arrangement was formalized through a bilateral agreement between the Canadian and United States Governments. This agreement contains three general provisions: the use of equivalent sanitary standards; occasional joint inspections; and a mechanism whereby the agreement may be terminated.

The rapid advances in freezing technology which took place after World War II made it practical for other shellfish-producing countries to ship frozen oysters, clams or mussels to the United States. Similarly, advances in air transport technology made it possible to ship fresh shellfish over considerable distances. Consequently, a number of countries, including Iceland, Mexico, Japan, the Netherlands, France, and Australia, have indicated an interest in exporting either fresh or frozen shellfish to the United States.

The technical and administrative problems involved in providing adequate sanitary control for shellfish imports were reviewed at the 1954 National Shellfish Sanitation Conference. The results of this discussion were summarized by Green.(1)

It was then concluded that the primary responsibility for further study of the problem rested with the Department of State. It is generally agreed in the United States that sanitary control of shellfish production is best obtained through the "control-at-source" principle utilized in the Cooperative Program. In contrast, the laboratory examination of samples of foreign shellfish on arrival at an American port of entry may not necessarily give satisfactory evidence as to the conditions under which the shellfish were produced and packaged. The problems involved in bacteriological examination are readily understood when it is realized that bacteriological standards which would be clearly indicative of pollution of growing or holding areas or insanitary plant practices have not yet been devised for domestically produced oysters. Unfortunately, the administration of a transoceanic control program comparable to that carried on between the United States and Canada appears to pose complex administrative problems and in many instances would not be economically feasible. The shellfish sanitation measures of the United States are not intended to function as a restraint of trade.

Since 1954 the importation of fresh or frozen shellfish has continued at an undetermined rate. Approximately 715,000 pounds of shellfish are now being imported into the United States, exclusive of Canada. (Table 1)

As previously reported, Canadian shippers are covered under the Certification Program in much the same manner as United States shippers. There is little question as to the sanitary quality of this Canadian product. Clam meats imported from Mexico generally enter the United States through the San Diego and Los Angeles custom districts. Conversations with the California State Department of Health have indicated that these clams are used in the production of heat-processed clam chowder. No apparent effort has been made to market these clams outside the State of California; hence the question of interstate certification has not arisen. In the case of Japan, a somewhat different situation prevails. Japanese clams have entered through the Los Angeles, Oregon, San Diego, San Francisco, and Hawaiian custom districts. The Public Health Service has no information as to the ultimate use of these clams. A more detailed tabulation of frozen clam imports by country of origin and entry by custom districts is shown in Table II.

There has recently been an active interest in both Mexico and Japan in increasing the volume of oyster shipments to the United States. A Corpus Christi, Texas, firm has proposed air shipment of shucked oyster meats from an oyster bed in the vicinity of Tampico, Mexico. A number of trial shipments have been made; however, the problem of marketing a non-certified product has apparently discouraged this operator. In Japan, a large, ultra-modern processing plant has been constructed to ship frozen oysters from the Hiroshima Bay area to the United States. Limited shipments of frozen oysters were made by this firm in the 1960-61 season. Recently, 80,000 pounds of oysters have been cleared through Seattle by the Food and Drug Administration.

Domestically, there is continuing interest in the development of a more logical system for Federal supervision of the sanitary quality of shellfish imports. This has been reflected in the adoption of resolutions by the Atlantic States Marine Fishery Commission, the Association of State and Territorial Health Officers, and the Conference of State Sanitary Engineers. These resolutions have all been aimed at the development of a rational Federal system for controlling the sanitary quality of shellfish imports.

The present system, in which the final decision as to the suitability of shellfish imports as a food product is left to the States, is clearly unsatisfactory. Foreign governments find it difficult to understand why a product admitted legally into the United States by the Food and Drug Administration should not be acceptable in any market in the United States. Similarly, those States with an active interest in shellfish sanitation have found it difficult to understand why shellfish could be admitted from foreign countries on the basis of only laboratory examination of the final product when such control measures have been demonstrated inadequate when applied to domestic production. There have, therefore, been numerous informal conferences between representatives of the Public Health Service, the Food and Drug Administration, the Department of State and, to a lesser extent, the firms interested in exporting oysters from Mexico and Japan.

The problem of sanitary control of oyster imports has also been considered from the legislative standpoint. Bills were introduced into the 86th Congress, 2nd Session, and the 87th Congress, 1st Session, which would have required the Public Health Service to make sanitary evaluations of foreign shellfish production, and which would have prohibited the importation of shellfish which had not been produced under adequate sanitary control. Neither of these bills were passed; however, it is known that there is a continuing interest in legislation which would provide an effective solution to this problem.

The Public Health Service is firmly committed to the philosophy that adequate control of shellfish production, harvesting, and packing is best achieved through a "control-at-source" program. Consequently, the Public Health Service cannot recommend the sale of fresh or frozen shellfish produced in any country other than Canada. Nor can the Public Health Service agree to the handling of such shellfish by shippers certified by States under the Cooperative Program.

The Public Health Service is interested only in assuring the sanitary quality of fresh or frozen shellfish offered for sale as a food product in the United States. There is no desire that the sanitary regulations of the Cooperative Program be used as a barrier to legitimate international trade. Conversely, the Service feels that it has no obligation to take or initiate steps which would increase the volume of foreign exports. It is our judgement that this is a responsibility of the Department of State. Therefore, it can be stated that while the Public Health Service will take no immediate steps to initiate development of a more logical system for the sanitary control of shellfish imports, we are ready to work with the representatives of the Department of State, and the foreign nations involved in the development of such a program. Similarly, the Service is willing to consider Federal legislation which would afford a mechanism whereby information might be obtained on the sanitary quality of shellfish offered for entry into the United States. It is, in fact, probable that Federal legislation affords the most desirable mechanism for dealing with this problem.

For the present, the responsibility for permitting the sale of shellfish from areas of unknown sanitary quality rests with the States and the local food control agencies.

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TABLE I

1960 IMPORTS OF FRESH OR FROZEN SHELLFISH

Country of Origin	Number of Pounds
Japan	504,267
Mexico	202,440
Portugal	4,070
Italy	2,756
Hong Kong	2,110
Spain	133
Sub-Total	715,776
Canada	337,898
Total	1,053,674

TABLE II

1960 IMPORTS OF FRESH OR FROZEN CLAMS

Country of Origin	Customs Distribution	Number of Pounds
Japan	Los Angeles	152,250
	Oregon	8,000
	San Diego	7,000
	San Francisco	238,335
	Hawaii	27,747
Mexico	Los Angeles	81,768
	San Diego	109,206
Portugal	New York City	4,070
Italy	New York City	2,756
Spain	New York City	133

APPENDIX D

THE SOUTH CAROLINA REPORT ON THE "HOT DIP" OR "SHOCK" METHOD OF OPENING OYSTERS

By

Somers B. Pringle
State Board of Health
South Carolina

South Carolina requests (we are taking the liberty here, of in addition including the state of North Carolina, with due apologies) that the method of opening raw oysters by first using the "Hot Dip" or what we call the "Shock" method be incorporated in the Shellfish Manual, Part II, certification be given on the same basis as is given to the other Plants,- not holding the "Shock" method of opening as an alien process.

At this point, it might be appropriate to explain just what we mean by the terms "Hot Dip" and "Shock". Traditionally to the Northeastern section of South Carolina and the adjacent area of North Carolina, oysters have been opened and shucked by immersion into iron pots of hot water, of varying degrees and for various lengths of time, to facilitate opening, under extreme insanitary conditions (at least in South Carolina). These oysters were opened on river banks, back yards or any place, put into glass fruit jars and bootlegged to the consuming public by door to door sales. As described later, this was called the Hot Dip by the Clam Industry. However, in order to get as far away as possible from any thought that this might be a cooked product, we in South Carolina called it a "Shock" to the oyster's complacency. These oyster shuckers, know of no other method of opening oysters, and would be at a complete loss as what to do, if they were called upon to crack the bill and insert the knife that way. In fact they might be termed stabbers, though they would not know what you were talking about, cracking irons or blocks are never used.

Confronted with this problem of bootlegging etc., South Carolina set out to legalize it, by first setting up plants or buildings and equipment that would meet interstate standards. We felt that if the process was carried out in a sanitary manner and in sanitary surroundings, we would be able to offer to the consuming public an acceptable product.

It might be assumed here, as to the exact reason this method of opening was originally used by this section. The oysters growing in this section were always of the cluster type (running some 500 to 600 to the gallon), the oysters being small, and the cluster tight, cracking the bill was difficult, further they are coated with heavy sticky mud. So we would further assume, that by soaking for an undetermined length of time, it removed some of the mud and the oyster cracked open for easy shucking. No thought was given then as to whether or not the oyster was semi-boiled.

The first step in our work after securing buildings and equipment, was the wash down of the oysters (most operators used a hose under pressure and get about 75% efficiency) to remove the mud, if we had had no other thought in mind, this would give us a better sanitary operation and in turn would give us a better end product, primarily, we also figured that a longer period of time would be required (or hotter temperature) to penetrate the mud, in order to accomplish our purpose, which was to relax the oyster. Frankly before we go any further into the premise regarding "shock" oysters let me make this statement that, 1) this is for the small plant operator, having from 1 to 10 shuckers. 2) It is for cluster oysters, running at best 4 pints to the bushel and for the operator dealing in this type of oyster that wants to get about 95% shuck out. It is not for the count trade or the half shell single type of oyster. These operators think it is faster work, no cracking time loss, no, shell chips to pick out on the skimmer, this time alone is a great savings. However, before you turn off your hearing aid, these operators turn out up to fifty gallons a day, some of their shuckers running five gallons or better in seven hours of work. The process is one that works best, if you keep only a small quanitity of shock oysters in front of the shucker, keeping a steady flow, rather than attempt to stockpile her. The operator at the shock tank must be on the job constantly, as there is not loafing time during shock periods. No attempt is made to shock long enough to open up the oyster, as we found this would allow the oyster to absorb the shock water, but shock just long enough to relax the oyster for easy stabbing and rapid shucking. In behalf of this method, first let us state, it is not new in the field of shellfish production. It appears that it was and maybe still a standard procedure in the Canadian Softshell Clam Industry, especially so, in the Brunswick and Nova Scotia area.

A Thesis for a Master's Degree in Science was presented by Mr. A. D. Tennant (1) in May 1949, entitled: "An Investigation of the Bacterial flora of the Softshell Clam (Mya Arenaria) in Brunswick and Nova Scotia." Within this Thesis Mr. Tennant presents several favorable factors and offers the same premise, that we had in mind all along, and I quote:

> "Several of the clam shucking plants inspected during the present studies followed the practice of dipping the washed shellstock clams in cauldrons of boiling or near-boiling water for periods up to twenty seconds. Following this treatment, the "shocked" clams were cooled by immersion in cold, fresh water, and shucked in the usual manner. This treatment causes the abductor muscle to relax, and the mantle becomes soft and loose facilitating easy and speedy shucking.
>
> The process was not carried out with any idea of reducing bacterial numbers; it was noted, however, that meats from clams treated in this manner showed a marked reduction in numbers of coliform bacteria and fecal stretococci. Sample of (1) shellstock, and (2) the same shellstock after the heat treatment were obtained wherever possible for testing." (Table XX)

The shocking of oysters as carried out in South Carolina, was not by any means intended to reduce coliform bacteria in any manner, however, if we did reduce it, that was another factor in our favor. Many persons have expressed an idea, that this shocking would cause changes in the oyster meat, well, we can state that in proper procedures as outlined below, we detect no physical changes in the appearances or changes in taste, and where offered for sale to the consuming public, they have been readily acceptable, and demand good.

We have for the past two seasons attempted to work on a formula of 150°F, dipping for a period of not more than two minutes in half bushel quantities, with an immediate chill down, with cold water (spray down, not immersion) after removal from the shock water. Oyster meat temperature taken just prior to chill down, ran from 92°F to as high as 103°F., chill down reduced this temperature to the high 70s to low 80s according to the temperature in the water supply, in cold weather with 60°F, pipe water, better results were obtained. We are now advocating the use of an additional source of ice cold water at the skimmer, to be used just prior to the pack, this is accomplished by either a tank in the ice house or by copper coils. Where installed, we are getting almost instant low temperature in the packing room. We are also attempting to have these installed in our standard type shucking plants. In one shock plant the water at the skimmer is about 38°F. The process presented both good and bad results based exclusively on the supervision of the process and adherence to good sanitation. Conditions which are synonymous to the operation of any shucking plant. As mentioned above, no apparent changes appeared in the meat. bacteriological samples were run on the meats at various stages of the process as well as on the shock water. It was found on samples taken in plants sticking closely to the formula and with two to three hour changes of the shock water, the bacteria count (coliforms) were within the limits (as described in the Proceedings - 1958 - (2) Shellfish Sanitation Workshop, page 11, for total bacteria per ml.) and we were interested to note that the pH value stayed constant during the seven day period of sub-sampling. This may have been due to the fact that all samples were held (check each day) at 36°F. to a high of 40°F. We feel, though I am most probably in error, that if oysters are kept on constant low holding temperature the pH will stay up.

Whether or not this is of any value, after repeated test between normal cold shucked oysters and "Shock" oysters, there appeared no difference whatsoever, in the pH of different batches of oysters tested. We wondered if the heat would change this, giving us some indication, so a laboratory test was run on two samples 1. cold shuck and 1. "shock" for eight days, the following table will give you some idea as to the keeping qualities. On the seventh day, both samples were allowed to go up to 76°F., and then refrigerated down to 40°F. The cold shuck dropped to pH 5.4 and the "Shock" to 6.0. From this we would assume the shelf of both was about the same. No appreciable odor was detected on either sample.

		COLD SHUCK		"SHOCK" SHUCK	
1st. day	pH	6.6	60F	6.6	80F
2-3-4th day		6.6	42F	6.6	42F
5-6th day		6.4	42F	6.4	42F
7th day	Temp raise		76F		76F
8th day	pH	5.4	40F	6.0	40F

Proper procedures and supervision were checked in the absence of the sanitarian by recording thermometers and in each case where time, temperatures and bath changes were recorded in proper sequence good bacterial results were secured, in meats and shock water, however, when the charts denoted, bad operation the MPNs and TPCs ran high and invariably this was in conjunction with high MPNs in the shock water.

As an example of inefficiency - Samples were taken in a plant operating at 128°F, pH on pickup of the samples was 6.8 - meat temperature 82°F. in the shucking bucket - packed refrigerated pint samples 41°F. - MPNs of 240,000 plus were recorded for six consecutive days. The pH ran from 6.8 on the pickup of samples to 6.6 on the fourth day and to 5.6 on the fifth and the same on the sixth. (see Table 1) Samples collected on the shock water were run and the results showed contamination, using a test (3) of five tubes of double strength Lactose Broth with 10 ml. of sample. Plate count test two plates on Agar using .1 ml. and, 01 ml. with a TPC of 1960. Here again this showed us that, we could expect no help from shock water of 128°F., in fact, this plant was allowing a shock time of at least five minutes, for relaxing the oyster muscle. This again proved to us the value of our trial of 150°F. for shock water, as we constantly secured negative samples from this type of shock water, where we had constant 150°F, plus, holding. We picked this temperature of 150°F., based on plant experience and on the long time pasteurization time of 145°F. for 30 minutes. We feel that, the process is a valid one, but one like any type of process should be operated under good supervision in good sanitary conditions.

The study made by the South Carolina Staff pointed the following suggestive requirements in the "Hot Dip" Plants. Temperature thermometers in shock water - clocks for timing the shock - daily record to be kept on time - temperature and shock water changes - requiring shock water change every two to three hours.

Let me at this point make one factor perfectly clear, so that there will be no doubts in your mind, this is N-O-T a process to use oysters from closed or polluted areas - this is no sterilizing procedure, all oysters opened in these plants are gathered from waters having a median of less than 70 per ml. as South Carolina has strict laws governing this. In fact, we do not allow oyster plants processing under the steamed canned method to process any oysters from waters having a median of more than 70 per ml. This 600,000 bushels breaks my heart, but under the law, it must be this way.

Conclusion being that: This recommendation is based on the fact, that the South Carolina State Board of Health has had this procedure under continuous experimentation for two years, and results have been such that they met all requirements of the State Board of Health, and is a popular procedure within the plants in which experimentation has taken place. Further on three occasions several representatives of the U. S. Public Health Service (from Washington and Atlanta) have observed this method here referred to and have indicated approval, in that, the plants using this method have, without exception, received relatively high ratings. The Shellfish Section, Division of Sanitary Engineering, with the approval of the proper authorities of the State Board of Health, strongly endorse the inclusion of this method as accepted procedure in the matter of opening oysters.

Finally the shock or hot dip being only a procedure within the processing and or shucking of raw oysters and should be incorporated as a part of Part II of the Manual or should be readily accepted by receiving states as a normal certification, if certified by the shellfish state of origin and meeting all the requirements of the Manual, as to source, buildings, equipment, and other procedures including listing on the PHS list of certified shippers, due to the fact that the certification co-operative program, is based solely on the acceptance of states certification (of those plants listed on the PHS listing), and the right is always reserved to the receiving states, to accept or reject the shipment if it does not meet the tentative proposed market standards as set up in the Workshop Proceedings.

In addition to the request for inclusion of the "Hot Dip" or "Shock" method of opening oysters, South Carolina would like to report that the State Health Officer, has issued a directive to the Shellfish Section as follows:

> "Any person dealing in shell stock shellfish from "open or approved" waters in South Carolina, shall have an established house or place of business conforming to all the sanitary requirements governing the sale of shellfish for human consumption and further no permitted certified shell stock dealer, may handle, store or pass through his plant, shell stock from "closed or polluted" areas, for any purpose whatsoever."

We fully realize or assume, that some states do have shell stock dealers without any established place of business or so we have been led to believe, such as boat to truck dealers, however, we have nothing to do with what another state may regard as a safe practice, but we do wish to inform those peoples who come from states permitting boat to truck dealers in shell stock for market, that under the above order from the South Carolina State Health Officer, if they operate in South Carolina, they will buy, or build a shell stock house and have one point of doing business, we cannot be assured otherwise. We have too much land adjacent to polluted waters, with oysters to constantly watch from the shore. We are not pointing any fingers, but these oysters are going into your states, and we desire to safe guard your people as well as ours.

All shell stock shippers handling oysters from "closed or polluted" areas for replanting and or purification, shall be required to secure a permit from the State Board of Health of South Carolina and in addition a permit for replanting and or purification from the receiving state.

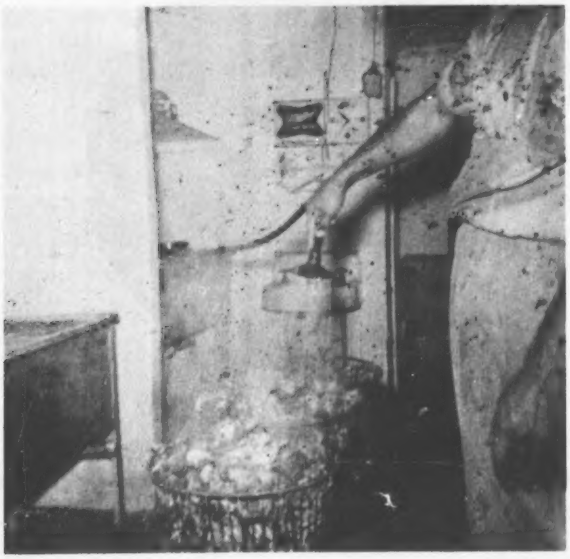
Further such permitted shell stock dealer, must establish within South Carolina one and one only point of truck loading and shipping. The shipment shall carry a manifest in duplicate copy to be signed by such state agency which has supervision over replanting and returned to the South Carolina State Board of Health, Shellfish Section, Columbia, South Carolina.

TABLE XX

REDUCTION IN FECAL BACTERIAL CONTENT OF SOFT SHELL CLAMS CAUSED BY COMMERCIAL "SHOCKING" PROCESS, 1947.

Source	Specimen	Treatment	Coliform MPN	Fecal Streptococci MPN
Jarvis & Co. St. Andrews N. B.	Clam (washed)	nil	1,100	2,400
	Shucked	"shocked" 30 sec. at 180°F, cooled, shucked.	230	130
Chamcook Packers Chamcook, N.B.	Clams (washed)	nil	1,300	1,700
		"shocked" 15 sec. at 190°F, cooled, shucked.	300	260
A. L. Baker & Son, Yarmouth N.S.	Clams (washed)	nil	1,100	330
	Shucked meats	"shocked" 20 sec. at 190°F, cooled shucked.	140 230	78 45
Northern Food Products, Grand Desert, N.S.	Clams (washed)	nil	9,200	780
	Shucked meats	"shocked" 30 sec. at 190°F, cooled, shucked.	100	230
Baker & Co., Ostrea Lake N.S.	Clams (washed)	nil	490	45
	Shucked meats	"shocked" 30 sec. at 180°F, cooled shucked.	20	0
General Seaboods, Ltd. Ostrea Lake, N.S.	Clams (washed)	nil	1,300	78
	Shucked meats	"shocked" 30 sec. at 180 F, cooled, shucked.	230	210
St. Andrews Harbour Station #4	Clams (washed)	nil	5,400	3,500
	Shucked meats	"shocked" 20 sec. (190°F) under lab. conditions.	790	330
St. Andrews Harbour Station #8	Clams (washed)	nil	5,400	3,500
	Shucked	"shocked" 20 sec. (190°F) under lab. Conditions.	330	230

COOL DOWN AFTER "SHOCK" IN HALF BUSHEL QUANTITIES



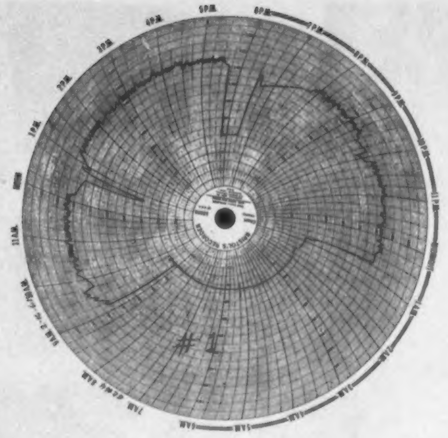


CHART NUMBER 1 DENOTES GOOD OPERATION OF "SHOCK". FAIRLY CONSTANT TEMPERATURE, ALONG WITH INDICATIONS OF CHANGE OF SHOCK WATER, THOUGH NOT QUITE ON FORMULA TIME. LABORATORY FINDINGS ON THIS PROCESS DAY DESIGNATED AS CHART 1.

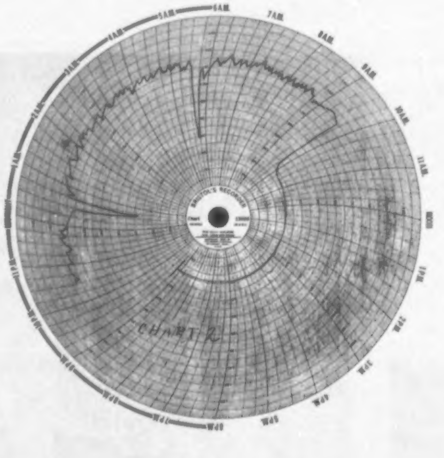


CHART NUMBER 2 WAS TAKEN AT ANOTHER PLANT DOING A GOOD JOB OF "SHOCK". HERE THE CHART WAS MOST PROBABLY PLACED IN THE RECORDER OFF POSITION, AS TO TIME OF DAY.

CHART I

SOUTH CAROLINA STATE BOARD OF HEALTH
SHELLFISH SECTION

SAMPLE NO: 37

PLANT PROCEDURE

SHOCK X

STANDARD

NAME OF PLANT ______ LOCATION Cherry Grove

Manager ______ Date 2-16-61

Source of Shell Oysters Murrells Inlet, S. C.

****

	Temperature	Time
Shock Water	150°	10:30
Chill down Water	64°	
Shock Oyster Meat	84°	
Chill Down Oyster Meat		
Shucking Bucket Meat	92°	
Fresh Packed Meat	72°	
Iced Down Meat	38°	
Chill Down Time to reach 45-50F. in Ice Box.		
Plant Air Temperature	64°	

Name of Oyster Consultant

______

Number must be same as Laboratory No.

Attached Inspection Sheet.

CHART 1

SOUTH CAROLINA STATE BOARD OF HEALTH
SHELLFISH SECTION

SAMPLE NO: 37

LABORATORY PROCEDURE

SHOCK X

STANDARD

NAME OF PLANT________ Location Cherry Grove Beach

Manager________ Date________ Time________

****

Sample Collection	Minutes	Temp	MPN	TPC
Shock Time				
Collected Sample Temp		38°		
Shucking Bucket Meat Temp		92°		
Shock Water Temp		150°		
Oyster Meat after Shucking		84°		
Oyster Meat off Skimmer		70°		
Oyster Meat Packed	2/17/61 pH 7.0	40°	1700	36,000
Sub-Sample Meat Held at 45°F. : 2nd Day	2/18/61	40°	1300	28,000
3rd Day	2/19/61	42°	330	26,000
4th Day	2/20/61	40°	1300	39,000
5th Day	2/21/61 pH 6.2	40°	820	31,000
6th Day	2/22/61	40°	4600	132,000
7th Day	2/23/61 pH 6.0	44°	1600	95,000

Shock water not contaminated
Neg.

Certified Shellfish Laboratory Man

____

PLATE 3

SOUTH CAROLINA STATE BOARD OF HEALTH
SHELLFISH SECTION

SAMPLE No:

SHOCK X

LABORATORY PROCEDURE

NAME OF PLANT Mobile Lab. Location Little River

Manager ____________ Date 2/17/61 Time ____________

****

Sample Collection	Minutes	Temp	MPN	TPC
Shock Time	5 +			
Collected Sample Temp		46°F		
Shucking Bucket Meat Temp		82°F		
Shock Water Temp		128°F		
Oyster Meat After Shucking		82°F		
Oyster Meat Off Skimmer				
Oyster Meat Packed	2/17/61 pH 6.8	41°F	240,000 +	T M T C
Sub-Sample Meat Held at 45°F. : 2nd Day	2/18/61 pH 6.8	40°F	240,000 +	T M T C
3rd Day	2/19/61 pH 6.6	42°F	240,000 +	
4th Day	2/20/61 pH 6.6	40°F	240,000 +	
5th Day	2/21/61 pH 5.6	41°F	240,000 +	
6th Day	2/22/61 pH 5.6	39°F	240,000 +	
7th Day	2/23/61 pH 5.6	40°F	240,000 +	

SHOCK WATER
5 Tubes Positive (coliform)
TPC 1960

Certified Shellfish Laboratory Man

____

PLATE 4

SOUTH CAROLINA STATE BOARD OF HEALTH
SHELLFISH SECTION

SAMPLE NO: 29

LABORATORY PROCEDURE

SHOCK X

STANDARD

NAME OF PLANT__________ Location Murrell Inlet

Manager__________ Date 2-4-61 Time 1:30

****

Sample Collection	Minutes	Temp	MPN	TPC
Shock Time				
Collected Sample Temp				
Shucking Bucket Meat Temp				
Shock Water Temp				
Oyster Meat After Shucking				
Oyster Meat Off Skimmer				
Oyster Meat Packed	pH 6.8		20	3,000
Sub-Sample Meat Held at 45°F. : 2nd Day		37°	130	12,000
3rd Day		38°	20	300
4th Day		35°	0	300
5th Day		38°	0	600
6th Day		40°	0	920
7th Day	pH 6.6	39°	0	1,400

Certified Shellfish Laboratory Man

____

SOUTH CAROLINA STATE BOARD OF HEALTH

POLLUTED OYSTER REPLANTING CONTROL PROCEDURE

1. Replanting shipper, shall first secure a permit to replant from the Receiving State, copy to be filed with the South Carolina State Board of Health.

2. Replanting shipper then shall secure a "P" pollution permit from the South Carolina State Board of Health.

3. Replanting shipper shall designate "ONE" point of loading and unloading of polluted shell stock acceptable to South Carolina State Board of Health and shall meet the requirements of the South Carolina Department of Labor.

4. Replanting shipper shall lists all trucks and/or other means of transportation including boats, barges, etc., with the State Board of Health as to type, make, state and/or Federal license number.

5. All permitted boats in closed and/or polluted areas shall have painted thereon a "P" permit number as described on the attached design.

6. All trucks and/or other means of transportation including boats, barges, etc., shall carry two - 30" X 18" yellow flags.

7. All trucks and/or other means of transportation including boats, barges etc., (where feasible) after loading to be sealed with the South Carolina State Board of Health wire seals or similar type.

8. All trucks and/or other means of transportation including boats, barges etc., shall have on board duplicate transit permits (issued by S. C. State Board of Health) filled out completely - one copy to be signed by proper authority in the Receiving State and returned to the S.C. State Board of Health within one week of date of shipment.

9. Telegrams to be sent to Receiving State to proper authority denoting number of trucks and estimated time of arrival at replanting point.

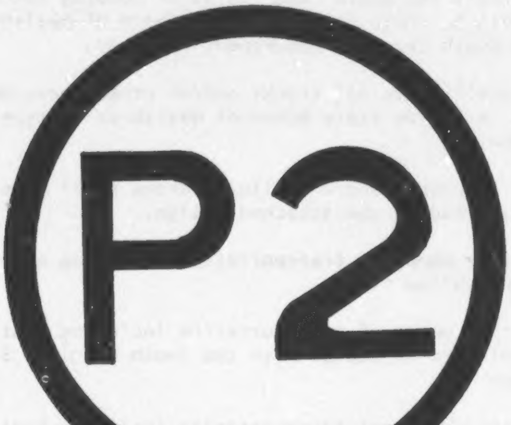
10. All picking and/or gathering boats in closed or polluted areas are to be under constant supervision while gathering and/or proceeding to unloading point.

11. Permitted Replanters are to gather or pick oysters from closed or polluted areas ONLY on designated days, as designated by State Board of Health. Collecting or gathering on other than designated days will be in violation regardless of having a permit. This is in view of the fact that gathering can only be done under direct supervision with limited personnel.

12. In the event of a breakdown or other emergencies by trucks or other means of transportation including boats, barges, etc., the operator shall notify the nearest local Health Department in the area in which the breakdown occurs for instructions as to the disposition of the shell stock.

DESIGN OF PERMIT TO BE PAINTED ON EACH SIDE OF BOAT GATHERING OR PICKING OYSTERS FROM "CLOSED" OR "POLLUTED" AREAS, ON THE STERN OF THE BOAT.

DESCRIPTION: AN EIGHT INCH CIRCLE, ONE INCH WIDE, IN BLACK OR WHITE ACCORDING TO BOAT COLOR. PERMIT LETTER "P" TO BE FOUR INCHES HIGH AND ONE-HALF INCH WIDE. PERMIT NUMBER TO BE OF SAME SIZE.



ANY BOAT SEEN GATHERING OR PICKING OYSTERS IN "CLOSED" OR "POLLUTED" AREAS, WITHOUT THIS PERMIT NUMBER IS SUBJECT TO ARREST. ALL GATHERERS AND/OR PICKERS ON PERMITTED BOATS, WILL CARRY IDENTIFICATION, AS TO NAME, AGE, RESIDENCE AND BY WHOM EMPLOYED.

EACH SHIPPER OF "CLOSED" OR "POLLUTED" OYSTERS FOR REPLANTING OR CLEANSING, SHALL FIRST SECURE A "P" TYPE PERMIT, BEFORE STARTING OPERATIONS.

SOUTH CAROLINA STATE BOARD OF HEALTH. DIVISION OF COMMERCIAL FISHERIES.

BIBLIOGRAPHY

(1) Tennant, A.D.: Master of Science Thesis, McGill University, Montreal, 105 pp, May 1949.

XX A.D. Tennant, Thesis, Table XX

(2) Proceedings - 1958 Shellfish Sanitation Workshop, page 11.

(3) S.C. Water Pollution Mobile Laboratory.

Temperature Charts, by S.C. Shellfish Section Personnel.

All samples were run under Laboratory Methods as described in "Shellfish Sanitation" - Syllabus - Part 3 - Supplementary Bacteriological Methods, page 14, Figure 1.

APPENDIX E

SANITARY AND QUALITY CONTROL OF THE CANADIAN SCALLOP INDUSTRY

by

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Department of Fisheries
Halifax, N. S.

In the beginning I wish to give a brief account of the size and importance of the Canadian scallop industry. The Canadian fishery is based entirely on a single species, the Giant or Sea Scallop, Placopecten magellanicus. The fishery falls naturally into two sections, the inshore fishery and the offshore fishery.

In 1960 there were about 33 draggers engaged in the inshore scallop fishery, the majority of them operating out of Digby, Nova Scotia. These draggers range from 45 to 60 feet in length. They carry a crew of 3 to 5 and land an average daily catch of 500 pounds of shucked scallops, although catches occasionally will exceed 1,000 pounds per day. Inshore scallop catches are normally shucked at sea, but occasionally draggers operating in the Gulf of St. Lawrence, will land catches in the shells.

The offshore scallop fishery in 1960 consisted of some 28 draggers. These range in length from 65 to 130 feet but the majority of them are in the 90 to 95 foot class. They carry a crew of 12 to 14 in the 65 to 80 foot class, and 18 to 21 in the 90 to 130 foot class. The draggers remain at sea up to 10 days in the fall, winter and spring, but during the summer, trips are shortened to 7 days. The offshore draggers land an average catch of 20,000 to 25,000 pounds. Practically all fishing is done on Georges Banks. All scallops are shucked at sea.

In 1960 the combined inshore and offshore fleets landed approximately 8 million pounds of shucked scallops. The inshore fleet landed about 1,600,000 pounds and the offshore fleet landed the balance 6,400,000 pounds.

Scallop draggers and scallop packing plants have been under sanitary control for a good many years. These controls were considerably strengthened in June 1961 when the Fish Inspection Regulations were amended to provide for the inspection of all export shipments of shucked scallops, which includes interprovincial shipments.

These regulations set forth requirements for the shucking, handling and packing of shucked scallops.

Holds, penboards and the dragger generally must be kept clean and sanitary at all times. All boats have toilet facilities and the crew must maintain personal cleanliness. The shucking pails and scallop wash boxes must be constructed of stainless steel. Shucked scallops must be washed in clean water and must not be exposed to pollution by any methods of handling. Sufficient crushed or flaked ice is carried to provide an icing ratio of one pound of ice to 1 pound of scallops.

All plants engaged in the packing and shipping of scallops operate under license. Therefore scallops can only be exported from a licensed plant which has been approved by an inspector, and which conforms to Sections 4.1.1.1 to 4.1.4.13 inclusive of Canadian Government Specifications Board, Specification 32-Gp-141A(1958). These sections lay down definite standards for construction of the plant, including floors, drains, walls, ceilings, ventilation, lighting, etc.; for plant sanitation, including toilet facilities, washbasins, hot and cold water, etc.; for plant operation, including an adequate supply of clean, safe, sanitary water of an M.P.N. of 2 or less, sewage disposal, offal or refuse disposal, a satisfactory rodent and insect control programme, prohibition against employing persons with communicable diseases or infections or "carriers," hand washing procedures and the wearing of proper type and clean uniforms, cleaning procedures for utensils, equipment, floors, etc.; and for plant equipment, including construction and type of materials used for tables, trays, containers, conveyor belts, etc.

The above standards are enforced by a field staff of fisheries inspectors. No persons can shuck or handle scallops for export without the prior approval of an inspector. Where scallops are packed for export in unbleached cotton bags, the inspector must examine the contents of as many bags as he feels necessary to arrive at a decision. If a proportion of the bags examined are found to contain scallops which do not conform to the requirements of the regulations, the inspector may require the emptying, culling, washing and re-packing of every bag in the shipment. These bags normally contain from 37 to 39 pounds of shucked scallops.

The regulations require scallop meats to be free of pieces of roe, gut, shell particles, sand and other foreign material. The meats must also be free from organoleptically detectable spoilage. Containers of scallop meats shall be packed for shipment in clean, finely crushed or flaked ice in amounts adequate to properly chill the contents.

APPENDIX F

SANITARY CONTROL OF THE MASSACHUSETTS SCALLOP INDUSTRY

By

Frederick C. Wilbour, Jr.
Division of Marine Fisheries
Boston, Massachusetts

The word "scallops" as defined by Massachusetts Law includes both the bay and the sea scallop. Total production during 1960 was 20,572,193 pounds, valued at $7,159,598. Heretofore, scallops were exempt from our shellfish laws, but, in 1961, the law was amended so that all scallops now come under our statutes or regulations. Quality control of this immense quantity of scallops is maintained by inspectors of the Division of Law Enforcement of the Department of Natural Resources acting on authority of Rules and Regulations promulgated by the Director of the Division of Marine Fisheries. These regulations apply from the time the scallops are harvested until their final acceptance by the consumer.

The sea scallop is found in offshore areas from New Jersey to Newfoundland, the greatest concentrations occurring on Georges Bank. From this area the sea scallopers of New Bedford take more than 73 percent of all landings of this species in the United States.

A sea scallop vessel is a medium sized craft averaging sixty-five to ninety-five feet in length, manned by a crew of ten or twelve men, and equipped with two ten- or twelve-foot dredges and the conventional fishing gear (gallows, winch, etc.) to handle the dredges. The distinctive features by which sea scallopers may be recognized are the two booms trimmed to hoist the dredges clear of the rail and the two shucking troughs located aft on either quarter.

The modern sea scalloper, fishing hundreds of miles from home, must necessarily keep its catch in the best possible condition, in order to receive top price. Taking into consideration the length of time required to catch, process, and deliver to the consumer a top quality product, let us consider some of the problems encountered in this effort.

Prior to sailing, the vessel takes on the necessary supplies, fuel, water and ice, and following the arrival of the captain and crew gets under way. Having left port, and steaming for ten to twenty-four hours, the scallop grounds are reached, the dredges lowered to the bottom, and fishing has commenced. While the first tow is being made, the hold man is busy below deck getting the pens ready to receive the bags of shucked scallops.

The twenty or thirty tons of crushed ice taken on in port is now put to use. To start with, about 12 inches of ice is shovelled into the bottom of the pens, in preparation to receive the catch. Meanwhile, the crew is preparing to handle the contents of the first tow. With these preparations in mind, the dredges are hauled to the gallows, the tackles from their respective booms hooked to the eye of the dredges, the dredges hoisted in over the rail and their contents dumped on deck - the journey to the consumer has started.

If the captain judges there are sufficient scallops to warrant fishing this area, a buoy is put over, the dredges are again set and the operation repeated day and night throughout the duration of the trip.

The deck watch cull the scallops from the trash picked up by the dredges, dump the culled scallops into the shucking troughs and begin the long job of cutting from each scallop the large abductor muscle, which holds the two valves or shells together. This muscle, known as the "eye," is the only part utilized for food, the viscera and shells being discarded and thrown overboard. The scallops are shucked into pails and when filled are dumped into the wash box containing running sea water.

When the sand and other foregin matter have been washed from the eyes, they are packed in unbleached cotton bags, holding about thirty-eight pounds, and packaged in ice in the previously prepared pens. Trips usually last from five to twelve days depending upon the weather and the quantity of scallops caught.

Upon returning to port, the quantity caught (known as the hail) is posted on the blackboard in the auction room where competitive bidding by the scallop dealers takes place. The catch is sold to the higgest bidder and the vessel proceeds to the pier where the successful bidder is located. Here the bags of scallops are taken from the ice in the vessels' hold, placed in canvas baskets, hoisted into the building, packed in sugar barrels for convenience of handling and stored in a cooler until they are processed or otherwise disposed of.

In 1958, the Division of Marine Fisheries received a request to conduct a study to determine the maximum length of time during which sea scallops would remain in satisfactory condition when packed in cotton bags and iced in the conventional manner. Accordingly, an expert fisherman working in conjunction with the Federal-State project aimed at improving the handling of fish at sea made a trip on the New Bedford sea scalloper "Aloha" on January 9, 1958.

Substantial samples of the first and last day's catch were taken for organoleptic, chemical and bacteriological tests ashore. These samples were iced in the conventional manner and immediately following the end of the trip were packed in barrels with crushed ice and stored in a commercial cooler at a temperature of approximately 40° F. A detailed report of the results of this test is attached to this paper.

Although the conclusions reached at the termination of this experiment must be regarded as tentative, there is strong indication that trips lasting more than ten days may result in the consumer obtaining an inferior product. The necessity of careful handling, washing and icing at sea cannot be overemphasized.

The scallop regulations for vessels engaged in the sea scallop fishery provide that holds and pen boards shall at all times be maintained in a sanitary condition. Ship personnel must use the toilet facilities provided aboard the vessels for personal cleanliness. Ice shall effectively separate all scallop bags from bulkheads, sheathing, shelves and pen boards and must be sufficient in quantity to last for the duration of the storage period.

All equipment of whatever kind, nature or description, whether within or without the establishment, fishing boat or vessel, used in the production, transportation, processing, storing, or packaging of sea scallops must be kept in a sanitary condition at all times. For instance, during the transition period, stainless steel wash boxes have taken the place of the old wooden boxes with the galvanized screening bottom; rusty, dented, galvanized pails have been replaced with stainless steel or plastic pails.

Heretofore, scallop bags have been stored in gear lockers under the whaleback with other equipment or in unused bunks aft or in the forecastle and so, a sanitary storage area is required in the new regulations.

Only new and previously unused bags which have been stored in a sanitary manner and protected so as to keep them free from contamination shall be used for sea scallops. Labeling of approved type containers shall bear the permit number of the producer and shall by by a method approved by the Director.

Departmental personnel discussed the proposed sanitary requirements for sea scallops with captains, boat owners, union officials and dealers and the effort has been on a voluntary basis for three years. With the enactment of Chapter 199 of the Acts of 1960 and the promulgation of the Rules and Regulations for sea scallops, certification will be mandatory after January 1, 1962.

Problems encountered in the bay scallop fishery are somewhat different.

The bay scallop fishery is unique in that in most cases a majority of those engaged in the operation are not fishermen! Throughout the years, tradesmen, farmers and local residents from all walks of life have looked forward to a vacation during the month of October, enjoying the crisp fall weather and the promise of making a quick dollar from the bay scallop fishery. Obviously, this cross section of our population do not have suitable facilities for shucking scallops under sanitary conditions.

With the opening of the bay scallop season on October 1st these men from all walks of life, in all types of nondescript boats from the skiff with a three-horsepower outboard motor to the expensive cabin cruiser start out to harvest their share of the bay scallop crop.

Except for the bay scallop grounds located off Wellfleet and Eastham in exposed areas in Cape Cod Bay most scallops are found in sheltered harbors and estuaries.

Bay scallops are caught with dredges which measure approximately thirty inches across the opening and have a twine back and a ring-link bottom. From two to four dredges are used depending upon the size of the boat and the available horsepower.

Arriving on the scallop grounds, short drifts are made until the greatest quantity of scallops are found, and then the boats try to stay in this area. Concentrations of boats dredging in a small area stir up the bottom often forcing mud and sand into the scallops.

Dredges are hauled in by hand and the contents dumped on a culling board where the scallops are picked out of the seaweed, shells and trash. As the dredges are dumped, they are returned to the water and the cycle repeated until the limit is obtained.

Massachusetts Law limits the individual's catch to ten bushels daily, but in most cases the cities or towns further reduce this limit to five bushels per man.

From this brief description of the activity on the water we may readily see that scallops washed free of mud and bottom sediments and put into clean bags which are stored above oily bilge water are important features of the bay scallop operation.

Having obtained his limit, the scalloper returns to shore and starts the long process of shucking the eyes from the scallop.

Because of the temporary nature of the fishery many types of shucking facilities are utilized, coal chutes set on barrels or oil drums, work benches in garages, cellars or sheds, culling boards blocked up against a building and any other conceivable means of holding loose scallops at a desirable height for shucking have been used. Many a long evening is spent "shucking out" after a day of fishing and at the beginning of the season a bushel of bay scallops will open about five pints. From the sanitation viewpoint it is this "shucking out" phase of the fishery that we are primarily concerned with.

The bay scallop regulations require that suitable facilities be provided for shucking operations. Floors must be constructed of impervious material and graded to drain quickly, walls and ceilings must be smooth and maintained in good repair, benches kept free of cracks so that they may be readily cleaned, waste containers provided for the viscera and shells, and all knives and other implements kept in a sanitary condition.

Water used in bay scallop washing or cleaning operations must be from a source approved by the Department of Public Health; toilet and lavatory facilities available; adequate refrigeration provided, and containers must be of an approved type.

Education of the public begins with the presentation of certain facts, in a simple and concise form, pertaining to a particular subject. For this reason, field men in the Department of Natural Resources have contacted fishermen and dealers and numerous public hearings have been held in an effort to acquaint the public with the purpose of this effort.

In summary, all aspects of the scallop regulations are interdependent, and when properly organized, will constitute a well rounded program for the sanitary control of the scallop industry.

SCALLOP DECOMPOSITION TEST

A series of scallops were taken from freezer at New Bedford and delivered by an inspector from Marine Fisheries. Sample #1 was shucked out on January 10, 1958 and sample #2 was shucked out on January 17, 1958. Bacteriologically, the samples were tested for total count with Difco Plate count media, incubated at 35°C. for 48 hours + 3 hours. Coliform count was determined with Desoxycholate Agar incubated at 35°C. for 24 hours + 3 hours. There was no indication of any growth of pathogenic enteries on the Desoxycholate agar. Series A was plated after shaking small pieces of scallops with the sterile, buffered, distilled water. Series B was plated after grinding the scallops in a Sterile Waring Blender. Chemically, the samples were tested for Trimethylamine Nitrogen and Amonia-Nitrogen ratio.

From the limited evidence gained in these tests, it would appear that the only index of value would be the T.M.A. Nitrogen. Using 7 as a standard, the scallops would appear to have a 14-day period between shucking and spoilage under the handling and storing conditions of this experiment. A lot more work would have to be undertaken before any definite results could be obtained.

(s) LEON A. LA FREENIESE

		SPC Col./g.	Coliform Count col./g/	NH3/N	TMA Nitrogen mgs./100 gs	Organoleptic
Jan. 20	IA	280	Less than 10	.141	.46	Odor Excellent
	IB	330	" " "			
	IIA	500	" " "	.092	.34	Odor Excellent
	IIB	700	" " "			
Jan. 22	IA	1,000	" " "	.157	Lab Accident	Odor Excellent
	IB	1,000				
	IIA	540	" " "	.139	Lab Accident	Odor Excellent
	IIB	540	" " "			
Jan. 24	IA	80	" " "	.258	3.0	Odor Fishy not bad
	IB	90	" " "			
	IIA	700	" " "	.167	.54	Odor slightly fishy
	IIB	680				
Jan. 27	IA	670	" " "	.285	7.0	Odor very strong
	IB	90	" " "			Fishy-Boiled turnip
	IIA	1,600	" " "	.219	3.0	Odor slightly fishy
	IIB	3,900	" " "			
Jan. 29*	IA	5,000	120	.309	7.5	Odor Bad-Gassy
*	IB	9,200	80			
	IIA	1,400	Less than 10	.226	4.0	Odor strong fishy
	IIB	1,900	" " "			
Feb. 3*	IIA	160,000	50	.239	7.4	Odor Bad-gassy

Samples marked with * had been handled at the freezer, explaining high counts.

APPENDIX FF

COLLABORATIVE STUDY OF SANITARY SIGNIFICANCE OF E.C. POSITIVE ORGANISMS IN SHELLFISH GROWING WATERS
(REPORT OF ACTIVITIES OF MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH)

by

Joseph A. McCarthy
Lawrence Experiment Station
Massachusetts Department of Public Health

Section I. Introduction

A. Summary

During the months of April through November 1959, sanitary surveys were made of the Merrimack River in Massachusetts, in Newburyport, and adjoining towns, and of the Essex River located in the town of Essex. During the surveys, sources of pollution were noted, a certain amount of hydrographical data were collected, and samples of shellfish and sea waters were collected for bacterial examination. A total of 16 sampling stations were set up, nine of which were in the Merrimack River, and seven in the Essex River. The location of these stations is shown in the attached map.

During the surveys the water temperature, the phase of the tide, and the direction of the wind were recorded. Chloride determinations were made on each sample. Water samples were collected both from the surface and at locations just above the bottom. Samples, whenever possible, were collected for three consecutive days in every month during the highest and lowest course of the tides. In all about 400 samples of sea water and 100 samples of shellfish were examined in parallel for coliform bacteria and for E. coli. Plate counts were also made on many of these samples.

In addition, during 1959 and 1960, E. coli determinations were made in parallel with routine coliform determinations on samples of sea waters and shellfish from all clean Massachusetts areas other than those in Essex, and from areas closed for shellfish taking. The clean areas are mostly on the South shore and Cape Cod. The closed areas include Boston Harbor and other harbors. The general character and particularly the sources and extent of pollution in both clean and closed areas have been determined by repeated determinations over many years.

B. Conclusions

As shown by the bacteriological data appended, the indicated numbers of both coliform and E. coli are quite in accord with the general character of the areas from which the samples were taken. On the whole the samples in the report give considerably higher numbers than those from other closed areas. The results from Essex and from other clean areas are almost identical. The comparatively few shellfish determinations from Newburyport on the whole give results much like those found over some 50 years of previous examination. In general the shellfish data from other closed areas have about the same relation to the figures from Newburyport as do the relative water samples. Also included the data for shellfish are bacterial results from some 82 quahog samples as a matter of comparison. Comments in detail include the following:

During certain times of the year the flow of the Merrimack River is sufficiently great so that the salinity is greatly reduced over the shellfish area. During these times bacterial results are always quite high. Very little of such data is included in this report. In the Essex River storm runoff from the numerous larger areas probably contributes comparatively few high bacteriological counts reported for the upper stations along this route.

The effect of seasonal variations is not very great in either water basin; with the possible exception of the month of June 1959, little if any effect of shellfish contamination was shown. Chloride determinations indicate no stratification in the Newburyport estuary at least during the summer months.

The ratios of E. coli to coliform have been determined for each of the samples reported. As might be expected, very high ratios were occasionally found. On the whole the number of coliform as compared to the number of E. coli was well within the first order to magnitude. It does not appear that the overall classification of any of the areas would be changed materially if the E. coli data were used instead of coliform data as an indication of the general bacterial content.

Section II. Description of Study

A. Merrimack River

The Merrimack River is the second largest stream in New England and has a total drainage area of 5,000 square miles of which 1,200 are within the Commonwealth of Massachusetts and 3,800 within the State of New Hampshire. It flows in a general easterly direction through several communities in Massachusetts and eventually empties into the Atlantic Ocean between the city of Newburyport and the town of Salisbury. Along its route of flow, it is joined by many tributaries.

Generally, all municipalities bordering the Merrimack River discharge raw sewage and industrial wastes into the river. The greatest amount of pollution is added to the river in the vicinity of Lowell, Lawrence, and Haverhill; the total population of these communities is in the vicinity of 208,000. In the Merrimack River estuary, the shellfishing grounds of the river, additional serious pollution is added by the city of Newburyport and the town of Salisbury with about 30,000 people.

Essex River

The Essex River is located entirely in the town of Essex and flows into the Essex Bay estuary which also receives waters from the Castle Neck River and various small creeks. The drainage area of this river complex is approximately 25 square miles. The Essex River has its origin in the lower swampy areas of Essex, various small streams, and Chebacco Lake which is on the border of the town of Essex and Wenham. The Essex River flows in a general northeasterly direction through the center of Essex and into Essex Bay which empties into the Atlantic Ocean between the town of Essex and Ipswich.

B. Maps showing watershed areas indicating points of significant pollution are attached.

C. Charts of areas under investigation indicating bacteriological sampling points are attached.

D. Sanitary Reconnaissance

In the Merrimack River, the sources of pollution are too numerous to outline and it should be sufficient to say that nearly all of the towns which border the Merrimack River discharge raw sewage and industrial waste directly into the river.

In the Essex River there are very few direct sources of pollution. One or two small malfunctioning subsurface disposal works were spotted during the reconnaissance of this area and recommendations were made to have them repaired. There is a great deal of boat traffic on and through this river, plus several small boat marinas. Intermittent pollution from these sources is undoubtedly present, even if in minor quantities. The marshy area surrounding this river undoubtedly contributes some organic and bacterial pollution due to wildlife habitation.

E. Hydrography

Merrimack River

During 1960, the Division of Sanitary Engineering of the Department of Public Health working in cooperation with the Division of Marine Fisheries of the Department of Natural Resources conducted a joint survey of the Merrimack River estuary. The objectives of this study are:

1. To relate the hydrography of estuary to the distribution of pollutants, and
2. To determine the degree of seasonal variation, and its effect on the contamination of the shellfish areas.

The volume of river flow into the Merrimack estuary is sufficiently high, averaging 7,210 cubic feet per second over a 10 year period, to cause marked changes in salinity throughout the estuary during a tidal cycle. It was found that the surface salinities near the mouth of the estuary of Plum Island may approximate that of coastal water (31.1/00) at high tide, but drop to less than 50 percent of this value at low tide. During freshet conditions, as in April, the water within the entire estuary may be virtually fresh. As might be expected, a definite negative correlation was found to exist between salinity and coliform concentrations. This obviously is a result of dilution through tidal mixing and perhaps to a lesser extent of the bactericidal effect of sea water. It was found that with the possible exception of the month of June, there was no apparent variation of shellfish contamination with season.

Essex River

The Essex River estuary (Essex Bay) is approximately 2,000 acres in size and except for two main channels and several minor ones, the bay reverts completely to tidal flats during the low phase of the tide. The mean range of tide is 8.5 feet with the spring range equaling 9.9 feet; based on the above figures it is estimated that with every change of tide in this estuary there is a change of approximately six billion gallons of water.

Chloride determinations obtained during the course of this study during various depths throughout the estuary indicate that there is no stratification in this estuary during the summer months at least.

Section III. Methodology

A. (a) Coliform determined according to Standard Methods, 11th Edition, using lauryl tryptose broth for presumptive and Brilliant Green Bile broth for confirmation. Incubator temperature in room, 35°C + 0.5°.

(b) *E. coli* determined using lauryl tryptose broth for presumptive EC medium for confirmation, at 45.5° in waterjacketed incubator.

B. Description of Sampling Methods and Equipment

Standard bacteriological sampling equipment was used during the course of the surveys. The surface samples were collected manually at a depth of approximately one foot below the surface of the water. The depth samples were collected at varying depths depending on the tidal conditions and were designed to collect water at a location situated approximately one foot from the bottom. For these samples, a bacteriological sampling cage was used and the sample bottle was allowed to fall swiftly to the designated depth where it was allowed to fill up and then hauled to the surface.

Section IV

BACTERIOLOGICAL DATA

	MPN/100 grams or ml					
Waters	Coliform			E. coli		
	10%	50%	90%	10%	50%	90%
Newburyport, all samples 233 samples	91	4,600	46,000	36	1,500	15,000
Essex Harbor 151 samples	3.6	23	460	3.6	9.1	93
All other clean areas 385 samples	3.6	23	460	3.6	9.1	140
Other closed areas, including Boston Harbor 199 samples	23	930	24,000	23	460	11,000
Shellfish						
Soft Clams						
Newburyport 56 samples	2,300	7,900	240,000	450	4,900	130,000
All clean areas, including Essex 56 samples	240	680	1,700	36	230	1,100
Other closed ares, including Boston Harbor, 211 samples	2,300	3,300	54,000	450	2,300	13,000
Quahogs or Hard Clams						
All areas 82 samples	< 20	< 20	490	<20	< 20	43
Sta. 1, all samples	15,000	46,000	460,000	4,300	20,000	75,000
top		46,000			24,000	
bottom		46,000			15,000	
Sta. 2, all samples	2,400	24,000	210,000	930	9,300	46,000
top		24,000			15,000	
bottom		24,000			4,300	
Sta. 3, all samples	2,400	24,000	110,000	930	9,300	46,000
top		35,000			9,300	
bottom		24,000			9,300	
Sta. 4, all samples	2,400	9,300	24,000	750	2,400	4,600
top		10,200			1,950	
bottom		9,300			2,400	
Sta. 5, all samples	2,400	4,600	23,000	230	930	4,600
top		4,600			930	
bottom		1,500			930	

	Coliform 10%	Coliform 50%	Coliform 90%	E. coli 10%	E. coli 50%	E. coli 90%
Sta. 6, all samples	36	930	4,600	36	230	930
top		930			230	
bottom		930			150	
Sta. 7, all samples	230	930	9,300	91	230	2,400
top		2,400			680	
bottom		750			91	
Sta. 8, all samples	36	280	7,500	15	230	2,400
top		580			230	
bottom		280			73	
Sta. 9, all samples	4,600	11,000	110,000	950	3,500	46,000
top		12,000			4,600	
bottom		11,000			2,400	
Average, all samples	230	4,600	46,000	36	1,500	24,000
Average, Stas. 1 - 4	4,300	46,000	110,000	930	9,300	46,000
Average, Stas. 5 - 8	43	930	11,000	36	430	2,400

Ratios of E. coli to Coliform — $\frac{\text{E. coli}}{\text{coliform}}$

Shellfish (soft clams) with coliform MPN/100 grams
Equal to or less than 2,300:

10%	.09	.01 to .09	18 or 16%
50%	.34	.10 to .24	20 or 18%
90%	1.00	.25 to .49	32 or 28%
		.50 to .99	20 or 18%
		1.00	22 or 20%

Shellfish (soft clams) with coliform MPN/100 grams
greater than 2,300:

10%	.12	.01 to .09	18 or 9%
50%	.36	.10 to .24	60 or 28%
90%	1.00	.25 to .49	59 or 28%
		.50 to .99	39 or 18%
		1.00	35 or 17%

Section V. Discussion of Data

A. Seasonal Variation

Seasonal variation apparently plays a very small part in the bacteriological density of the waters of both the Merrimack River estuary and the Essex River. As stated previously, excepting for a short time during the year, conditions in the Merrimack River remain substantially the same, i.e., highly polluted.

B. Influence of Rainfall on Storm Runoff

There is no question about the influence of rainfall on storm runoff in the Merrimack River. During certain times of the year, the river water is entirely fresh due to the large influx of freshwater into the river from drainage areas. During these times, when the dilution of waste material in the river by ocean water is lacking, the bacteriological results may be quite high.

In the Essex River, storm runoff from the numerous marshy areas could conceivably raise the coliform density. This may be a partial answer to the relatively high bacteriological counts obtained at the stations located in the upper reaches of the river.

C. The data have been plotted in the attached probability curves. They indicate that on the whole the numbers of coliform and the numbers of E. coli tend to be quite parallel. They also indicate, as stated above, that these numbers very seldom differ from each other by a whole degree of magnitude.

MERRIMACK RIVER SURVEY - JUNE OCTOBER, 1959
SAMPLING STATIONS
SEA WATER

STA. #1: At Groveland Street Bridge, between Groveland and Merrimack.

STA. #2: Bridge Street Bridge at West Newbury, Haverhill and Merrimack.

STA. #3: At Merrimack-Amesbury line.

STA. #4: At Deer Island Bridge in Amesbury.

STA. #5: At Railroad Bridge in Newburyport.

STA. #6: East of Woodbridge Island in Newburyport.

STA. #7: The basin in Newbury.

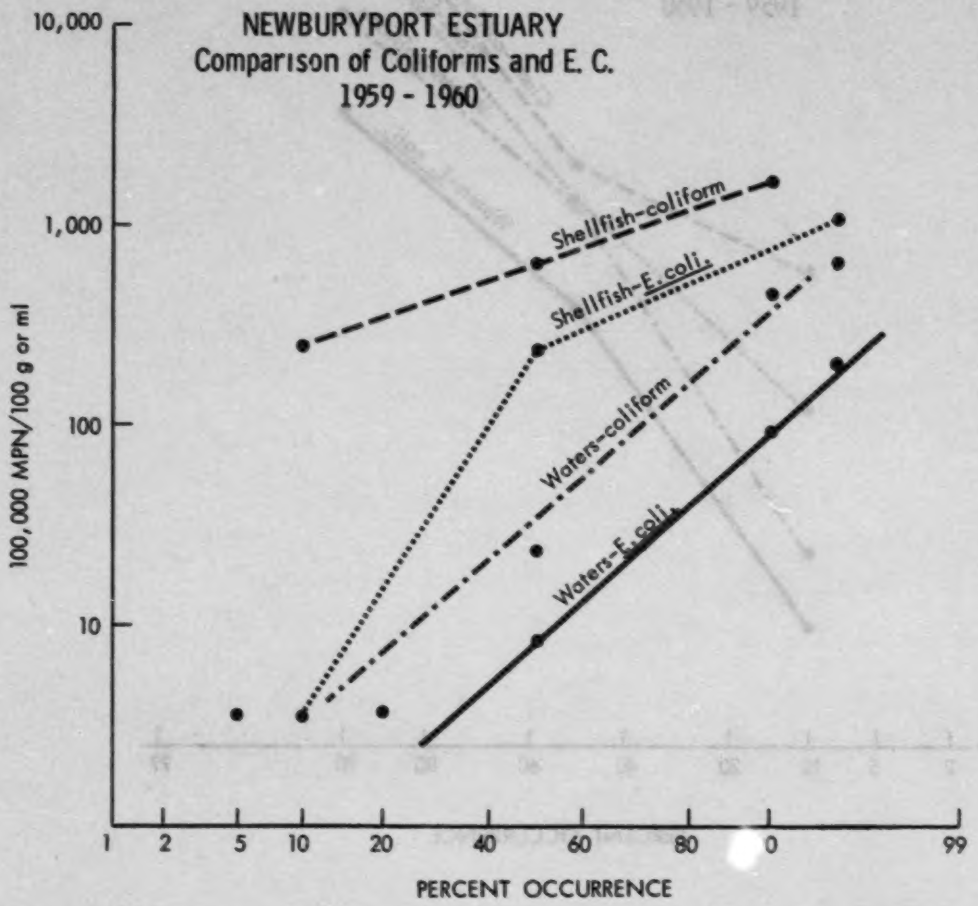
STA. #8: Plum Island Point.

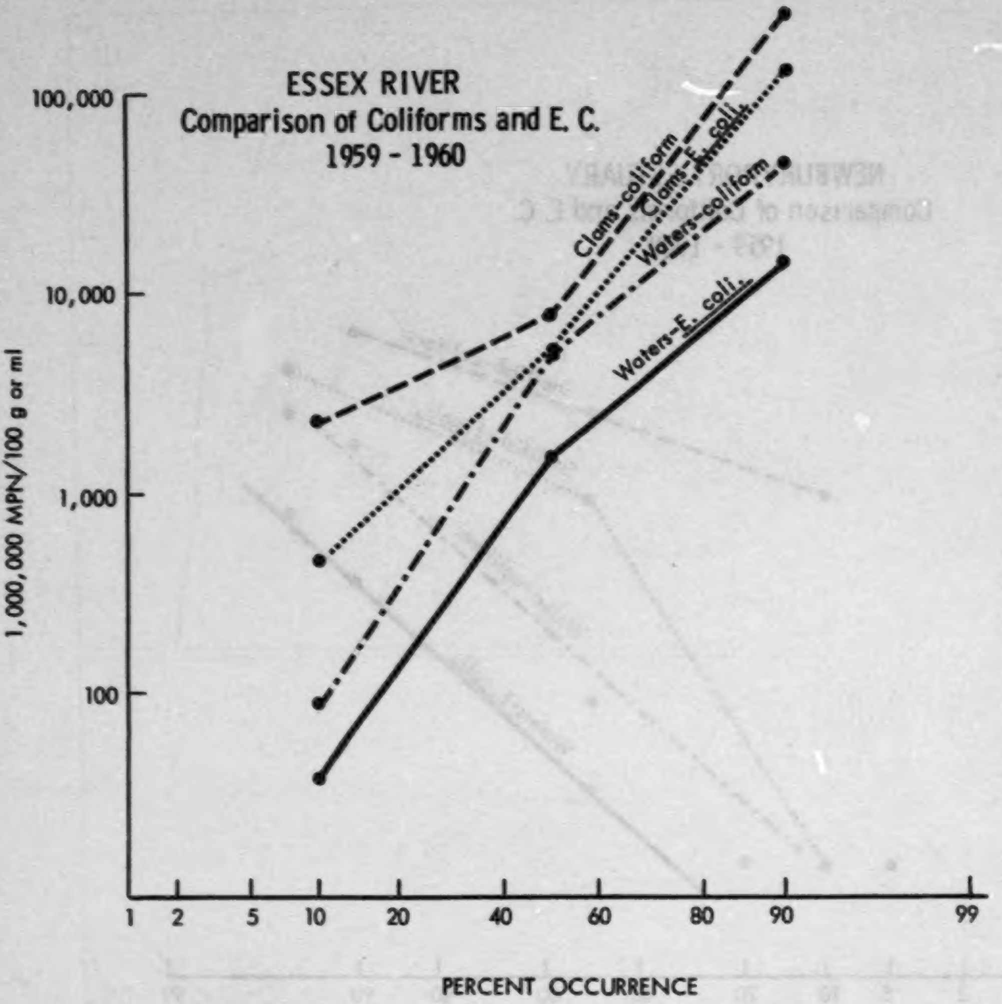
STA. #9: Pow-Wow River - Amesbury.

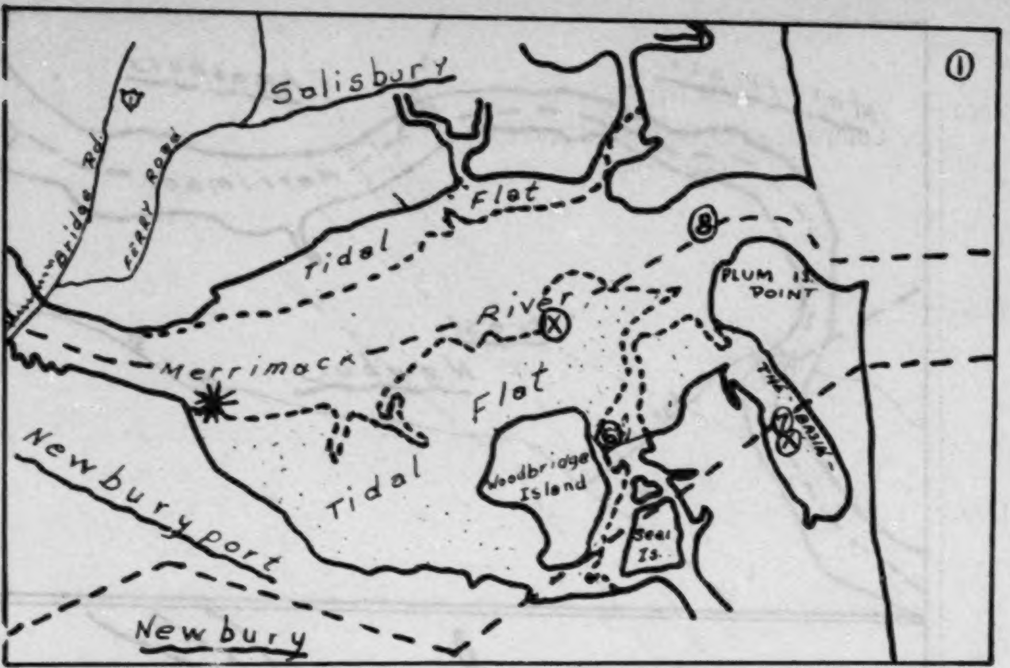
SHELLFISH

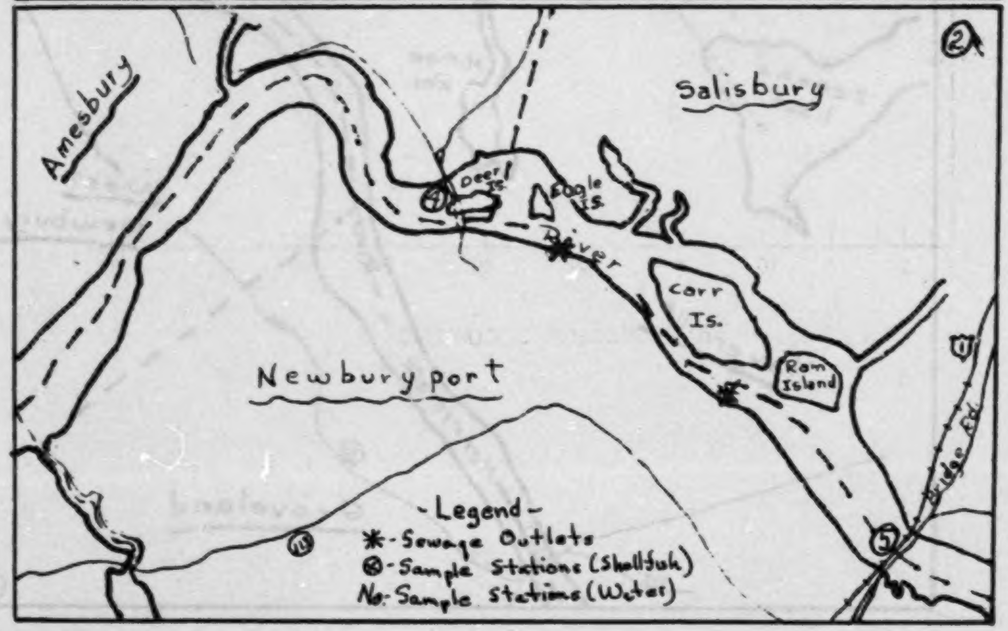
The Basin

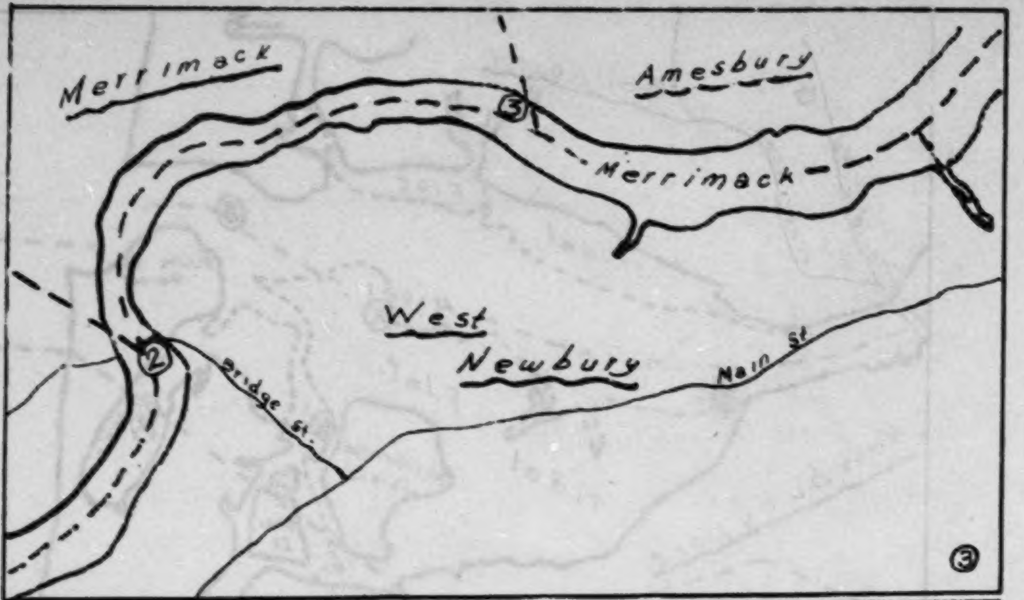
Joppa Flats - North of Woodbridge Island

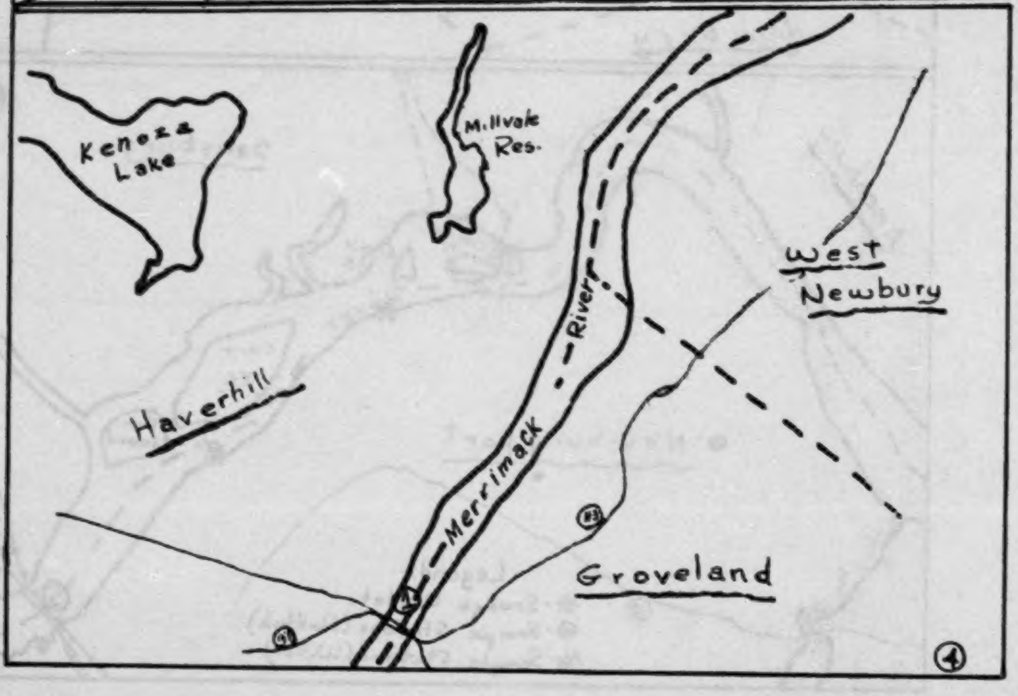

NEWBURYPORT ESTUARY
Comparison of Coliforms and E. C.
1959 - 1960
10,000
1,000
100
10
100,000 MPN/100 g or ml
Shellfish-coliform
Shellfish-E. coli.
Waters-coliform
Waters-E. coli.
1
2
5
10
20
40
60
80
0
99
PERCENT OCCURRENCE

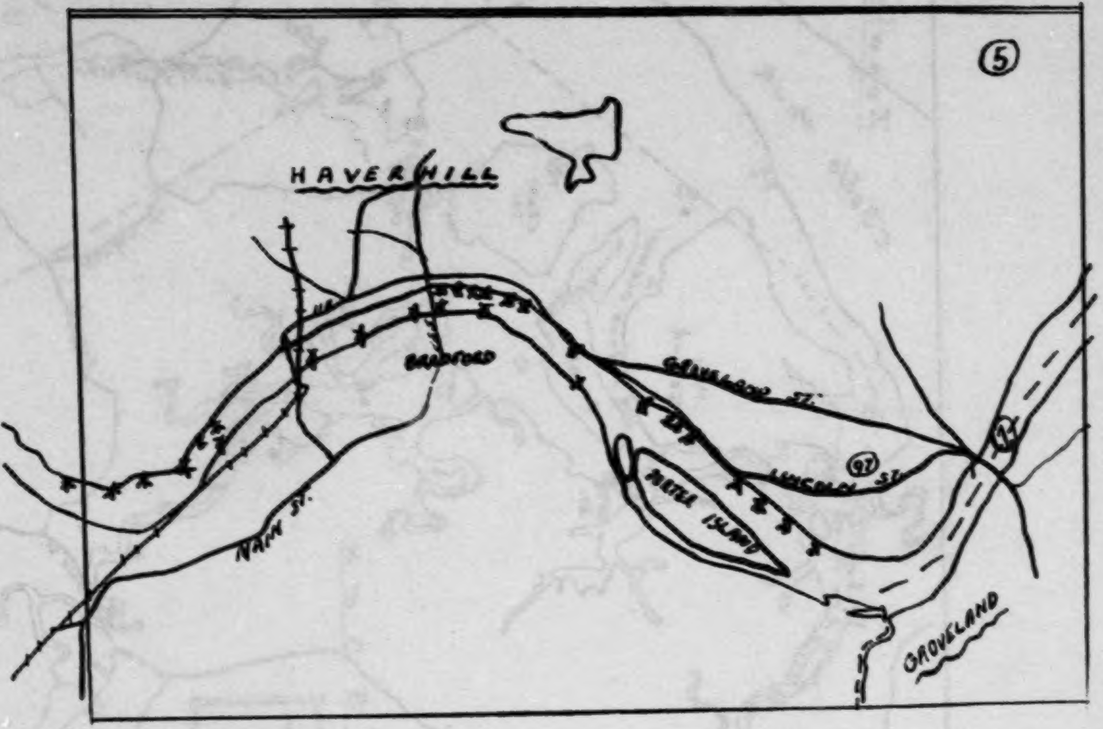

ESSEX RIVER
Comparison of Coliforms and E. C.
1959 - 1960
100,000
10,000
1,000
100
1,000,000 MPN/100 g or ml
Clams-coliform
Clams-E. coli
Waters-coliform
Waters-E. coli
1
2
5
10
20
40
60
80
90
99
PERCENT OCCURRENCE

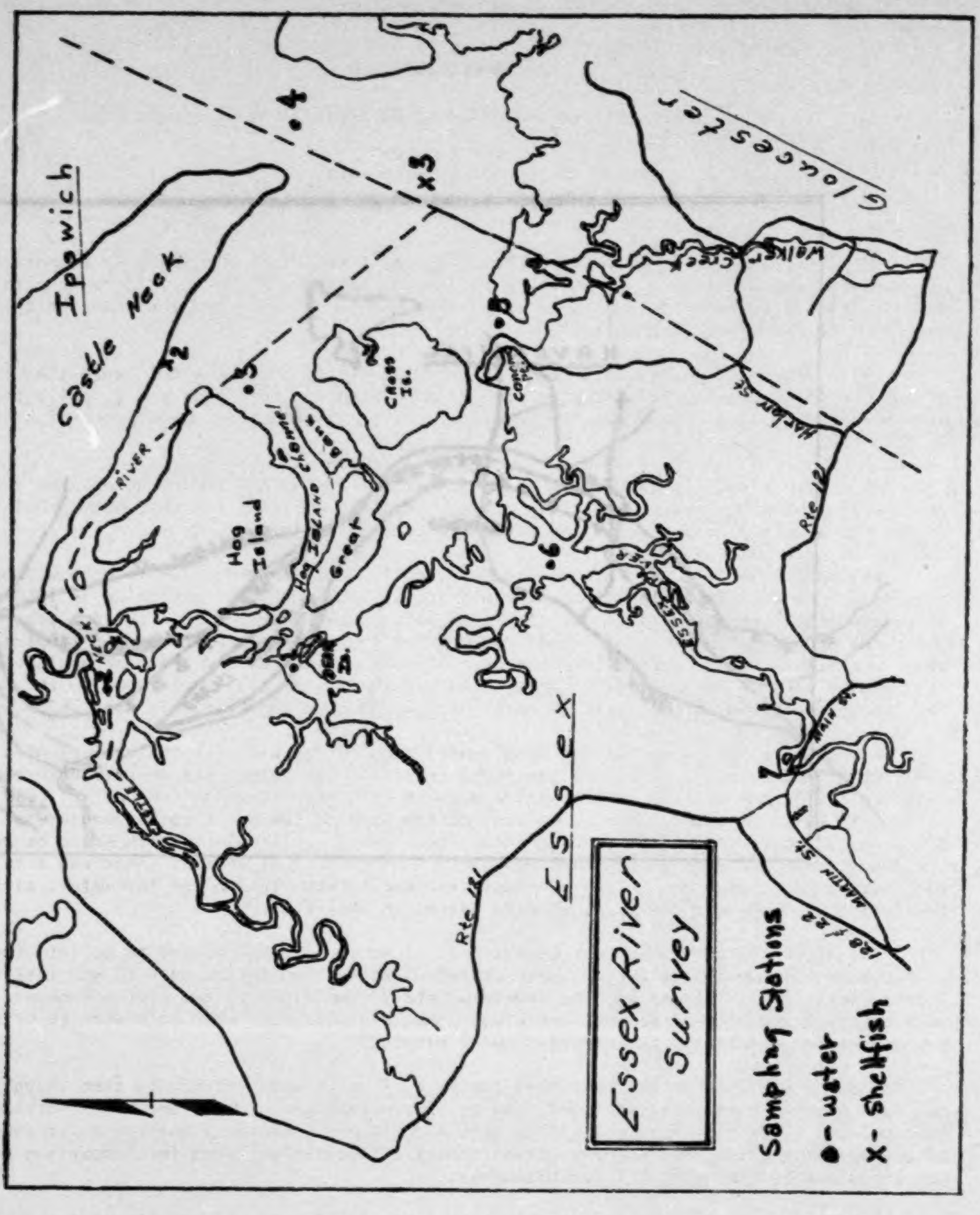

①
Salisbury
1
Bridge Rd
Ferry Road
Tidal Flat
8
Plum Is. Point
Merrimack River
Tidal Flat
The Basin
7
6
Woodbridge Island
Seal Is.
Newburyport
Newbury


②
Amesbury
Salisbury
Deer Is.
4
Eagle Is.
River
Carr Is.
Ram Island
1
Newburyport
Bridge Rd
5
10
- Legend -
✱ - Sewage Outlets
⊗ - Sample Stations (Shellfish)
No. - Sample Stations (Water)


Merrimack
Amesbury
③
Merrimack
West Newbury
②
Bridge St.
Main St
③


Kenoza Lake
Millvale Res.
West Newbury
Haverhill
River
Merrimack
Groveland
④


⑤
HAVERHILL
BRADFORD
GROVELAND ST.
97
LINCOLN ST.
MAIN ST.
ISLAND
97
GROVELAND


Essex River Survey
Sampling Stations
●- water
X- shellfish
Ipswich
Castle Neck
Gloucester
Essex
Hog Island
Great Bank
Hog Island Channel
Cross Is.
River
Essex River
Walker Creek
Harbor St.
Rte 121
Main St.
Martin St.
128 & 22
1
2
3
x3
4
5
6
7

APPENDIX G

COLIFORM AND E.C. DETERMINATIONS

Robert M. Brown
Chief, Bureau of Environmental Health
Maryland State Department of Health

Mr. Chairman, fellow members of the Workshop, I report on our study in summary as it has not yet been written up. I am sorry that we do not have a copy available to give you. Our results are so recently available to us that we have not yet had the opportunity, as we want to talk them over in the family

With regard to our study, when Dr. Perry and his laboratory group began the procedure of our shellfish waters, providing us with data on both the coliforms and E. coli, these values have been a matter of record over these years. In terms of actual performance of a study, we date back at least 10 years.

As a result you might say of a rash of restrictions we had to impose on some small but relatively productive oyster producing areas, there was a clamor for what many people contended was a more needed direct inspection of fecal pollution.

Having available to us information on E. coli. determinations, we thought we would proceed to get this into shape that we could study. Consequently, beginning in 1950--and it continues at present--all of our shellfish water survey data is punched each year into IBM cards according to the area involved, the station, water temperature, air temperature and the coliform and E. coli determinations. Over these 10 years we have accumulated some 16,000-plus records of water examination. As you might appreciate, this got to the point that we wondered how in the world we were going to do anything with this data which we had.

Approximately nine months ago Gene Jensen came to our aid with the proffer of a computer study which would enable us to analyze these records. Our objectives were to determine what relationship if any existed in our region between coliform determinations and E. coli determinations in these waters. Since we on many of the samples taken had corresponding shellfish sample data, we had shellfish results in coliform and E. coli values which would be available for comparison. We wanted to determine by means of this study whether there was a relationship between coliforms and E. coli in shellfish and between the values for water, either coliform or E. coli and the corresponding values in shellfish.

The 16,000 records were then classified. I would not want to try to go into the detail of the manner in which the records were prepared, other than to indicate to you that the indeterminately high values and the indeterminately low values we got eleven thousand-plus, both coliform and E. coli values were eleven thousand plus, and when both were reportedly zero these for purpose of our analysis we eliminated.

Also, I would point out that when the E. coli value was determinate then though the coliform was not these were included, and by appropriate grouping by the folks setting up the computer study they were classified into logarithmic groupings, which made it possible to accomplish the analysis and the establishment of regression lines for comparison of the two organisms by the method of least squares.

By virture of the fact that we had such a large group of values, it was possible to obtain results which reflect considerable confidence in the relationship.

The method of analysis indicates that within the range of significance represented by our data that the predictability of E. coli identity from an MPN coliform is generally good but does admit the possibility of error between determinations. The conclusion which it has been possible to draw from this study is that there is a direct and predictable relationship between the coliform value and the E coli value in the waters subject to this study. I will indicate to you the specific nature of that in a moment.

Although there is an apparent relationship between coliform and E. coli in shellfish, since we did not have but 500 samples to give weight to this analysis, the relationship cannot be said to have statistical significance. There was no relationship in the values between the coliforms or E. coli in the water and the coliforms and E. coli in the shellfish. In other words, we could not predict from the values of the water what would be in the shellfish, or vica versa.

By far the bulk of the samples which were analyzed were from areas of satisfactory quality of producing water. As is the case though in the taking of all samples we have some high and some low from which we strike the medium, so that the thing that interested us most was the kind of result which we might expect ot have in this relationship that would give us a more positive indication of the quality of the water.

We, of course, want to use the method of determination which is, on the one hand, the best to do, the most significant, the most reliable, and which will lead us to the best possible classification of our producing waters.

I want to indicate to you that in the analysis the study was performed by grouping the samples into four categories as to water temperature. We had a category from 35 to 50 degrees, 51 to 65 degrees, 66 to 80 degrees, and over 80 degrees. These are Fahrenheit temperatures. The results by years within this 10-year span were grouped into 6 periods, in order to produce a reasonably comparable number of samples in each one of the time periods analyzed.

There was little variation in the results, coliform and E. coli, as compared one to the other between year periods selected in the study and between the temperature ranges involved, except that there did seem to be some falling off in the amount of coliform and of E. coli determined in the temperature range of over 80 degrees.

Therefore, we have an analysis based upon comparison between not only annual periods or grouped time periods over a 10-year span, but between temperature ranges as well.

This leads us to the following indication of comparison between coliform and E. coli results. If you want to note these you might list under a coliform MPN per 100 ml. and E. coli MPN per 100 ml. the following figures:

Under coliform 35, and the corresponding value of E. coli, 8; coliform 70, E. coli 14; coliform 700, E. coli 68. This is practically a straight line relationship and would make it possible to predict the E. coli value from the coliform determination. We have not yet determined the confidence with which from this particular regression line we might predict the coliform from E. coli.

We hope to utilize this information in the future to determine the significance of variation between regions with regard to higher coliform or E. coli values in shellfish grown in waters which are of a satisfactory MPN, either coliform or E. coli-wise, where we have apparently a trend of higher shellfish values as we proceed southward and loweras we proceed to the north. When I say "we" I am thinking of this from the standpoint of the Workshop rather than Maryland, because we do not have this variation within our own region, but this as we know is one of the regional difficulties which has confronted the kind of control we have been interested in exercising.

I would like to acknowledge in this study the assistance of our laboratory and staff people in doing the laboratory work, in keeping the records, and our gratitude to Mr. Jensen, Dr. Harris, and Mr. Austin of the Sanitary Engineering Center in carrying out this computer study. I hope that we will have this in a more definite form that can be made available to you in the near future.

APPENDIX H

COLIFORM E. C. GAS POSITIVE RELATIONSHIPS IN SHELLSTOCK, SEA WATER AND BOTTOM SEDIMENTS FROM AREAS OF VARYING BACTERIAL QUALITY

By

J. A. Hope
and
C. W. Wiley
Virginia State Department of Health
Bureau of Shellfish Sanitation

Section I - Introduction

A. Purpose of Study

Although new coliform standards for shucked oysters were adopted at the 1956 "Shellfish Sanitation Workshop," it was recommended that study be initiated to try to determine the validity of the coliform test. Further, it was suggested that perhaps other organisms might serve better as an indicator organism. Therefore, a comprehensive study was outlined using the coliform group and E. coli with results to be reported to the 1958 Shellfish Sanitation Workshop.

At this meeting a standard was set up of bacteriological criteria for market oysters employing fecal coliform organisms or E C gas positive organisms as the indicator organism. This, of course, was quite encouraging to those of us that had been actively engaged in the study, "Cooperative Study to Evaluate Present Bacteriological Criteria of Market Oysters." Since certain members of the coliform group are found free living in nature in contrast to the isolation of E. coli only from the fecal sources of warm blooded animals, it began to appear that we had an effective tool. No doubt, there are many places throughout the country that have areas with a high coliform MPN and low pollution from warm blooded animal sources.

It was recognized that much information was needed prior to application of this test to other segments of the oyster industry, for instance, production level and growing areas. This need was borne out when we experienced rather high seasonal results during the fall of the 1958 oyster season. As a result Virginia undertook a study to further evaluate E. coli and to try to determine coliform - E C gas positive relationships.

Section II - Description of Study Areas

For the purpose of this study, areas selected were designated as "excellent" "good" and "polluted." The areas "excellent" and "good" are both state approved for the direct marketing of shellfish - naturally the polluted area oysters were unacceptable for direct marketing.

The "excellent areas" selected were far removed from concentrated sources of human and industrial pollution with a low population density. The "good areas" were chosen to represent areas subject to occasional pollution and of higher population density. The "polluted areas" were picked to represent areas subject to frequent contamination with raw and treated sewage effluent and population densities typically metropolitan.

All samples in this study were collected from the lower 1/3 of Chesapeake Bay or its tributaries. Chesapeake Bay is 170 nautical miles in length exhibiting rectilinear currents averaging 15-18 miles in width and with a mean tide of 3', a spring tide of 3.6' at the mouth. The most distant sampling location, Stingray Point, has a mean tide of 1.2' a spring tide of 1.4'.

The "excellent" areas New Point and Stingray Point are 20 and 37 miles above the Hampton Roads area respectively.

The "good" areas Hampton Flats and Lynnhaven are located in Hampton Roads and 15 miles below Hampton Roads respectively.

The "polluted" areas are in the Elizabeth River which flows into Hampton Roads and Little Creek 10 miles below Hampton Roads.

THE EXCELLENT AREAS

At the Newpoint Comfort sample location, 1¼ miles off shore in 20' of water, the bottom characteristics are hard and sandy. The land area is rural with one marina type facility and three negligible sources of animal pollution.

At the Stingray Point sample location, 3/4 of a mile offshore in 15' of water the bottom characteristics are hard sandy clay. This land area is rural with eight sources of questionable pollution, two marina type facilities, three sources of animal pollution (insignificant), 11 residences with faulty pits or drain tile systems.

THE GOOD AREAS

The Hampton Flats sample point, one mile offshore in 10' of water, has hard sandy bottom characteristics. There are municipal areas along the North Shore of Hampton Roads. Sources of pollution are: one municipal terminal, one municipal sewage treatment plant, four sewage pumping station, four marinas.

The Lynnhaven sample point, one mile from mouth was on high sand bars. Basically, the area is rural with heavy development on the upper reaches of the stream. The sources of pollution consist of four sewage treatment plants with secondary treatment, nine septic tanks with sand filters contributing effluent to stream, three small marina type installations, four animal pollution.

THE POLLUTED AREAS

The Elizabeth River sample point, in the midst of the harbor was 200 yards offshore in 8' of water having muddy bottom characteristics. The number of major sources of pollution are: four raw sewage, four sewage treatment plants, nine septic tanks and four marina type facilities.

The Little Creek samples were collected from the bank of stream about 15 yards from the high water mark. The bottom was muddy. The number of major sources of pollution are: three raw sewage, one sewage treatment plant, five spetic tanks, six marinas.

SECTION III - METHODOLOGY

In our study two different areas representing each classification "excellent" "good" and "polluted" were sampled over a period of approximately one year. During the hot weather season 2-3 sets of samples were collected each month and 1-2 during the cold weather season. By combining results from areas of the same classification type, a total of 40 samples each of "shell stock" "sea water" and "bottom sediment" were examined from the "excellent" and "polluted" areas and 39 from the "good" area. Past studies have indicated a seasonal break in our growing areas which is correlated to the sea water temperature - above 50°F. April - November and below 50°F. December - March. In the seasonal breakdown 32 of our samples were of the hot weather season.

The time lapse between the collection of samples and setting up varied from three to six hours - a maximum. The routine coliform and SPC tests were incubated in a standard water jacketed incubator at 35°C. ± 0.5°. In the E C gas positive test we employed a covered water bath with forced circulation at 44.5°C. ± 0.2°. The average reading of the temperature during the period of this study of morning, noon, and evening readings (500) is 44.46°C. with a minimum (one observation) of 44.0°C. and a maximum of 44.9°C. (one observation). A certified thermometer was used to correct our routine thermometer.

In the refrigeration of samples when atmospheric temperatures were above 50°F, samples were iced or an abundance of canned refrigerant was used.

Shell Stock

In four of our areas the shell stock was collected by a small hand dredge. The other two were hand collected directly from the exposed bottom.

Shell stock was handled as outlined in the "Collaborative Survey of Bacteriological Condition of Market Oysters."+ Samples were scrubbed, allowed to drain, aseptically shucked, blended and inoculated.

Examinations performed were the coliform, the E C gas positive test (a presumptive test) and the standard plate count. The five tube method was used in the case of the shell stock and the three tube method for the sea water and bottom sediments based on precedents set in previous studies and routine work. The dilutions were varied from one gram to 0.001 gram portions.

Sea Water

These collections were grab samples within one foot of the surface. In two of the areas occasionally the tide had ebbed off 20-30' from the oysters, however, the water sample was collected anyway.

These samples were shaken as indicated in standard methods and inoculated. Examinations performed were the coliform and E C gas positive test - 3 tube method. The dilutions were varied from 10 cc to 0.001 cc.

Bottom Sediments

The bottom sediments were collected in several different ways:

1. Bottom samples (3 areas) - A sterilized bottle was thumb screwed into one end of a pipe, with the opposing end considerably expanded. The device is pulled by rope and roots into the bottom.
2. Dredge collection (1 area) - Mud was collected from dredge as oysters were collected.
3. Direct collection (2 areas) - The sample bottles were pushed along the bottom by hand until a sample was obtained. Supernatant liquid was always decanted prior to weighing 10 grams and adding to 90 cc. sterile buffered water. Examinations performed were the coliform and E C gas positive test - 3 tube method. The dilutions were varied from 1 gram to .001 of a gram.

+ "Proceedings of 1958 Workshop"

SECTION IV BACTERIOLOGICAL DATA

TABLE I

SHELL STOCK COLIFORM AND E C GAS POSITIVE MPN AT 10, 50 and 90 PROBABILITY PERCENTILES

	Excellent Area		Good Area		Polluted Area	
PERCENTILE	COLIFORM	E C	COLIFORM	E C	COLIFORM	E C
DECEMBER, JANUARY, FEBRUARY, MARCH						
10	-20	-20	26	-20	225	74
50	-20	-20	67	33	1,700	230
90	800	-20	125	88	11,400	710
No. of samples	8	8	8	7	8	8
APRIL, MAY, JUNE, JULY, AUGUST, SEPTEMBER, OCTOBER, NOVEMBER						
10	-20	-20	60	-20	1,700	300
50	82	-20	610	81	14,000	2,250
90	460	100	6,200	900	120,000	16,500
No. of samples	32	32	31	31	32	32
ALL SEASONS						
10	-20	-20	25	-20	720	145
50	63	-20	340	58	9,000	1,300
90	450	72	4,500	680	105,000	12,000
No. of samples	40	40	39	38	40	40

NOTE - means less than

TABLE 2

SEA WATER COLIFORM AND E C GAS POSITIVE MPN AT 10, 50 and 90 PROBABILITY PERCENTILES

	Excellent Area		Good Area		Polluted Area	
PERCENTILE	COLIFORM	E C	COLIFORM	E C	COLIFORM	E C
DECEMBER, JANUARY, FEBRUARY, MARCH						
10	-3.6	-3.6	-3.6	-3.6	210	-36
50	-3.6	-3.6	5.6	-3.6	960	170
90	-3.6	-3.6	55	15	4,250	2,600
No. of samples	8	8	8	8	8	8
APRIL, MAY, JUNE, JULY, AUGUST, SEPTEMBER, OCTOBER, NOVEMBER						
10	-3.6	-3.6	-3.6	-3.6	330	-36
50	-3.6	-3.6	9.4	-3.6	3,600	700
90	42	-3.6	110	40	39,000	21,000
No. of samples	32	32	30	30	31	32
ALL SEASONS						
10	-3.6	-3.6	-3.6	-3.6	210	-36
50	-3.6	-3.6	7.4	-3.6	2,600	610
90	21	-3.6	8.4	35	34,000	13,000
No. of samples	40	40	38	38	39	40

NOTE - means less than

TABLE 3

BOTTOM SEDIMENT COLIFORM AND E C GAS POSITIVE MPN AT 10, 50, and 90 PROBABILITY PERCENTILES

	Excellent Area		Good Area		Polluted Area	
PERCENTILE	COLIFORM	E C	COLIFORM	E C	COLIFORM	E C
DECEMBER, JANUARY, FEBRUARY, MARCH						
10	-36	-36	-36	-36	750	83
50	-36	-36	370	70	5,200	360
90	94	--	5,900	260	35,000	3,150
No. of samples	8	8	8	7	8	8
APRIL, MAY, JUNE, JULY, AUGUST, SEPTEMBER, OCTOBER, NOVEMBER						
10	-36	-36	36	-36	800	44
50	38	-36	440	51	5,800	1,100
90	1,050	46	2,400	700	43,000	27,000
No. of samples	32	31	31	30	32	32
ALL SEASONS						
10	-36	-36	38	-36	800	55
50	-36	-36	300	60	5,800	1,100
90	68	40	2,400	570	42,000	22,500
No. of samples	40	39	39	37	40	40

NOTE - means less than

TABLE 4

SHELL STOCK COLIFORM AND E C GAS POSITIVE MINIMUM, MEDIAN, AND MAXIMUM MPN

	Excellent Areas		Good Areas		Polluted Areas	
MPN RANGE	COLIFORM	E C	COLIFORM	E C	COLIFORM	E C
DECEMBER, JANUARY, FEBRUARY, MARCH						
Minimum	-20	-20	-20	-20	330	110
Median	-20	-20	94	45	2,850	280
Maximum	490	20	140	78	16,000	790
No. of samples	8	8	8	7	8	8
APRIL, MAY, JUNE, JULY, AUGUST, SEPTEMBER, OCTOBER, NOVEMBER						
Minimum	-20	-20	-20	-20	20	-20
Median	150	-20	460	78	16,500	2,400
Maximum	2,400	490	160,000	54,000	+240,000	35,000
No. of samples	32	32	31	31	32	32
ALL SEASONS						
Minimum	-20	-20	-20	-20	20	-20
Median	130	-20	330	78	9,200	1,300
Maximum	2,400	490	160,000	54,000	+240,000	35,000
No. of samples	40	40	39	38	40	40

NOTE - means less than
+ means more than

TABLE 5

SEA WATER COLIFORM AND E C GAS POSITIVE MINIMUM, MEDIAN, AND MAXIMUM MPN

	Excellent Areas		Good Areas		Polluted Areas	
MPN RANGE	COLIFORM	E C	COLIFORM	E C	COLIFORM	E C
DECEMBER, JANUARY, FEBRUARY, MARCH						
Minimum	-3.6	-3.6	-3.6	-3.6	240	-36
Median	-3.6	-3.6	6.3	-3.6	1,015	240
Maximum	-3.6	-3.6	43	9.1	24,000	2,400
No. of samples	8	8	8	8	8	8
APRIL, MAY, JUNE, JULY, AUGUST, SEPTEMBER, OCTOBER, NOVEMBER						
Minimum	-3.6	-3.6	-3.6	-3.6	230	-36
Median	-3.6	-3.6	9.1	3.6	4,600	780
Maximum	460	43	11,000	4,600	240,000	110,000
No. of samples	32	32	30	30	31	32
ALL SEASONS						
Minimum	-3.6	-3.6	-3.6	-3.6	230	-36
Median	-3.6	-3.6	9.1	3.6	2,400	460
Maximum	460	43	11,000	4,600	240,000	110,000
No. of samples	40	40	38	38	39	40

NOTE - means less than

TABLE 6

BOTTOM SEDIMENT COLIFORM AND E C GAS POSITIVE MINIMUM, MEDIAN, AND MAXIMUM MPN

	Excellent Areas		Good Areas		Polluted Areas	
MPN RANGE	COLIFORM	E C	COLIFORM	E C	COLIFORM	E C
DECEMBER, JANUARY, FEBRUARY, MARCH						
Minimum	-36	-36	91	36	930	91
Median	-36	-36	330	91	4,600	930
Maximum	91	91	4,600	230	24,000	2,400
No. of samples	8	8	8	7	8	8
APRIL, MAY, JUNE, JULY, AUGUST, SEPTEMBER, OCTOBER, NOVEMBER						
Minimum	-36	-36	-36	-36	36	-36
Median	62	-36	430	82	10,100	1,015
Maximum	4,600	91	92,000	4,600	+240,000	+240,000
No. of samples	32	31	31	30	32	32
ALL SEASONS						
Minimum	-36	-36	-36	-36	36	-36
Median	40	-36	430	91	9,200	930
Maximum	4,600	91	92,000	+24,000	+240,000	+240,000
No. of samples	40	39	39	37	40	40

NOTE - means less than
+ means more than

TABLE 7

ALL SEASON EC/C RATIOS

EC/C RANGE	EXCELLENT AREA	GOOD AREA	POLLUTED AREA
Minimum	-.01	.02	.03
Median	.26	.32	.12
Maximum	1.00	1.00	1.00
No. of samples	15	34	34
SEA WATER			
Minimum	.02	.16	-.01
Median	.24	.50	.32
Maximum	.46	1.00	1.00
No. of samples	4	24	38
BOTTOM SEDIMENT			
Minimum	.02	-.01	-.01
Median	.40	.21	.18
Maximum	1.00	1.00	1.00
No. of samples	7	27	35

NOTE - means less than
+ means more than

TABLE 9

SEAWATER % ATTAINMENT OF COLIFORMS MPN

MPN	EXCELLENT AREAS	GOOD AREAS	POLLUTED AREAS
COLIFORM	DECEMBER, JANUARY, FEBRUARY, MARCH		
-70	100	100	0.0
+230	0.0	0.0	100
No. of samples	8	8	8
APRIL, MAY, JUNE, JULY, AUGUST, SEPTEMBER, OCTOBER, NOVEMBER			
-70	90.5	83.2	0.0
+230	3.1	6.7	98
No. of samples	32	30	31
ALL SEASONS			
-70	92.5	87.0	0.0
+230	2.5	5.2	97.2
No. of samples	40	38	39

NOTE - means less than
+ means more than

TABLE 8

SHELL STOCK % ATTAINMENT OF
COLIFORMS AND E.C GAS POSITIVE MPN

	Growing Areas		
MPN	EXCELLENT AREAS	GOOD AREAS	POLLUTED AREAS
COLIFORMS	DECEMBER, JANUARY, FEBRUARY, MARCH		
-230	100	100	0.0
230-2,400	0.0	0.0	50
+2,400	0.0	0.0	50
No. of samples	8	8	8
E.C GAS +			
- 78	100	100	0.0
+230	0.0	0.0	50
No. of samples	8	7	8
	APRIL, MAY, JUNE, JULY, AUGUST, SEPTEMBER, OCTOBER, NOVEMBER		
COLIFORMS			
-230	62.5	19.4	3.1
230-2,400	37.5	51.6	9.4
+2,400	0	29.	87.5
No. of samples	31	31	32
E.C.GAS +			
- 78	87.5	51.5	2.5
+230	3.1	32.3	97
No. of samples	32	30	32
	ALL SEASONS		
COLIFORMS			
-230	67.5	35.9	2.5
230-2,400	32.5	41	17.5
+2,400	0	23.1	80
No. of samples	40	39	40
E.C. GAS +			
-78	90	60.5	2.5
+230	2.5	26.3	87.5
No. of samples	40	38	40

NOTE - means less than
+ means more than

Section V - Discussion of Data

In the discussion to follow, it will be well to have before us market standards which have been in use by the receiving areas:

*Coliform	+	**E. C.	
Class I	0 - 16,000	Class IA	0-78
Class II	16,000 - 160,000	Class IB	79-230
Class III	±160,000	Class II	±230

In table I we have the shell stock, coliform and E. C. gas positive MPN at the 10, 50 and 90 probability percentile for the cold, hot, and all seasons samples. Immediately it will appear that the hot weather samples are weighted.

While all these tables reveal interesting information for brevity and more representative results we will stick closely to the all seasons samples. Most of us will agree that the median deals with a lower percentage of our results than we can justify. Our thoughts should include a greater percentage of our results in other words.

In table I, the "All Seasons" "Excellent" areas, the E. C. gas positive results of the shell stock at the 90 percentile level approaches the cut off with a MPN of 72. For the "Good" area this figure jumps to 680. The "Hot" weather season bears this out also as the E. C. median of the good areas reads 81.

In table 8, our percent attainment for coliforms and E. C. gas positive MPN of the shell stock we readily see that 60.5 percent of our E. C. gas positive samples from the "Good" areas are 78 or less, however, 26.3% were greater than 230. The "Hot Weather" season reflects an even worse condition with 32.3% ± 230. Approximately 50% of our oysters are harvested during the hot weather season.

It is noteworthy from table 9, the percent attainment of coliform MPN of the sea water, that these approved areas easily comply with the standard used by regulatory agencies of a coliform MPN of 70/100 ml. In the case of the "good" area 87% were 70 or less and only 5.2% were +230. There is little difference even for the hot weather with 83.2 percent -70 and 6.7 +230 and the superior results for the cold weather samples 100% -70 (good area).

Therefore, it is immediately obvious that at the growing areas we are not complying with the interim E. C. market standards.

In table 2 we have the Coliform and E. C. gas positive MPN at the 10, 50, and 90 probability percentile for the sea water. It is immediately obvious that all seasons, hot and cold at the 50 percentile for the approved areas are of quite superior quality with respect to the coliform. They are good in all areas and seasons except for the 90% of the "good" area for the all and hot season. But what is even more interesting is the fact that at all percentile levels of the all, hot and cold weather seasons, for the excellent area the E. C. gas positive MPN is -3.6. The same is true at the 50% level for the "good" area.

This indicates that the E. C. gas positive test is too insensitive in its present form for routine use in the examination of sea water which is of "Excellent" quality.

Table 3 gives us the coliform and E. C. gas positive MPN at the 10, 50, and 90 probability percentiles for the bottom sediment. This is offered purely on an informational basis since there is no standard method for its collection and examination.

+ Coliform and E. C. results on MPN/100 ml
* 1956 Workshop
- Less than

** 1958 Workshop
- Greater than
+ Greater than

In table 7 we have the all season E. C. gas positive/coliform ratios. It is interesting to note in the case of the shell stock that there is a greater rate of recovery of E. C. gas positive organisms from the "Excellent" and "Good" areas as compared with the polluted areas. This is also true of the sea water for the "Good" area.

This bears out the work of Dr. Tennant in Canadian waters.

The standard plate count results were satisfactory at all levels from the "Excellent" and "Good" areas.

CONCLUSIONS

We therefore conclude:

1. The E. C. gas positive MPN, the fecal coliform density, of 0-78 as market criteria for shucked oysters is too stringent.
2. The coliform and E. C. gas positive populations in oysters, sea water and bottom sediments are parallel. The E. C. gas positive test appears to have an advantage of a more realistic reportable result. A question remains - does this realism outweigh the ease and facility of the coliform test.
3. The E. C. gas positive results of the sea water from our "Excellent" areas are so low as to indicate that the test is too insensitive for practical use in its present form.

REFERENCES

1. A. D. Tennant, Ph. D. and others. An Evaluation of the E. C. (44.5°C) Confirmation Test for the Estimation of Escherichia coli Densities in Seawater and Shellfish.
2. C. J. Velz, Graphical Approach to Statistics.
3. U.S.P.H.S. Proceedings 1956 Shellfish Sanitation Workshop.
4. U.S.P.H.S. Proceedings 1958 Shellfish Sanitation Workshop.

TABLE A (MR. HOPE AND MR. WILEY)
RAW DATA

	Shell stock			Mud		Sea water	
	Coliform MPN	E. coli MPN	SPC	Coliform MPN	E. coli MPN	Coliform MPN	E. coli MPN
Excellent							
Sting Ray Point	68	-20	320	460	36	9.1	3.6
New Point	-20	-20	-300	62	-36	93	43
Good							
Lynnhaven	160,000	54,000	57,000	92,000	9,200	11,000	4,600
Polluted							
Little Creek	240,000+	24,000	56,000	9,200	470	24,000+	1,500
Elizabeth River	240,000+	22,000	58,000	9,200	860	11,000	1,100
The above samples collected October 23,1958. On October 21, 2.82" of rain fell.							
Excellent							
Sting Ray Point	78	-20	50	91	-36	3.6	-3.6
New Point	230	-20	1,000	-36	-36	-3.6	-3.6
Good							
Lynnhaven	5,400	330	330	11,000	43	43	23
Hampton Bar	330	230	140	2,400	430	3.6	3.6
Polluted							
Little Creek	17,000	2,400	9,100	4,600	930	1,500	240
Elizabeth River	24,000+	5,400	6,700	4,600	930	2,400	2,400
The above series collected on October 27, 1958.							
Excellent							
Sting Ray Point	45	20	-300	-36	-36	-3.6	-3.6
New Point	-20	-20	300	-36*	-36	-3.6	-3.6
Good							
Lynnhaven	230	45	-3,000	430	91	23	3.6
Hampton Bar	2,400	1,300	7,900	430	230	23	23
Polluted							
Little Creek	5,400	2,400	-3,000	36	-36	2,400	43
Elizabeth River	9,500	2,800	-3,000	930	430	460	240
The above series collected on October 29, 1958.							
Excellent							
Sting Ray Point	20	-20	390	-3.6	-3.6	-3.6	-3.6
New Point	-20	-20	370	230	91	-3.6	-3.6
Sample Numbers 970-N, 975-N inclusive Salinity 6.7%							

NOTE 3 grams light of 10
- means less than

TABLE A (MR. HOPE AND MR. WILEY)
RAW DATA

	Shell stock			Mud		Sea water	
	Coliform MPN	E. coli MPN	SPC	Coliform MPN	E. coli MPN	Coliform MPN	E. coli MPN
Good							
Lynnhaven	260	-20	280	230	91	9.1	9.1
Hampton Bar	130	78	310	430	-36	93	-3.6
Sample Numbers 967-N, 969-N and 976-N - 978-N Specific gravity 1.016 Salinity 7%							
Polluted							
Little Creek	9,200	640	3,000	2,400	430	11,000	30
Elizabeth River	5,400	1,300	2,500	240,000+	46,000	430	230
Sample Numbers 961-N - 966-N inclusive specific gravity 1.014 Elizabeth River.							
The above samples collected on November 10, 1958.							
Excellent							
Sting Ray Point	230	78	-300	-36	-36	9.1	-3.6
New Point	410	-20	-300	150	-36	-3.6	-3.6
Good							
Lynnhaven River	230	78	-300	430	91	-36	-36
Hampton Bar	330	130	-3,000	36	-36	14	-3.6
Polluted							
Little Creek	1,700	490	3,000	24,000	930	230	91
Elizabeth River	3,500	1,100	-3,000	11,000	930	930	230
The above samples collected on November 11, 1958 and November 20, 1958.							
Sample Numbers 999-n - 1007-N and 1047-N - 1050-N							
Excellent							
Sting Ray Point	20	20	-300	-36	-36	-3.6	-3.6
New Point	-20	-20	-300	91	36	-3.6	-3.6
Sample Numbers 1171-N - 1176-N inclusive December 10, 1958.							
Good							
Lynnhaven	78	45	520	930	73	9.1	9.1
Hampton Bar	110	45	830	4,600	210	43	7.3
Sample Numbers 1137-9 December 8, 1958 - Samples Numbers 1177-N - 1179-N December 10, 1958.							
Polluted							
Little Creek	2,200	170	3,200	4,600	2,400	430	230
Elizabeth River	4,600	490	3,700	4,600	430	930	430
Sample Numbers 1131-N 1136-N December 8, 1958							
Excellent							
Sting Ray Point	-20	-20	430	36	-36	-3.6	-3.6
New Point	130	-20?	1,400	73	-36	-3.6	-3.6
Good							
Lynnhaven	110	20	200	91	36	3.6	3.6
Hampton Bar	78	20?	700	2,400	20+?	-3.6	-3.6

TABLE A (MR. HOPE AND MR. WILEY)
RAW DATA

	Shell stock			Mud		Sea water	
	Coliform MPN	E. coli MPN	SPC	Coliform MPN	E. coli MPN	Coliform MPN	E. coli MPN
Polluted							
Little Creek	3,500	110	6,500	4,600	930	24,000	240
Elizabeth River	330	130	-3,000	11,000	930	2,400	2,400
All of above samples collected on January 26, 1959.							
Results are questionable where indicated due to a portion of the sample being discarded.							
Excellent							
Sting Ray Point	490	-20	-3,000	-36	-36	-3.6	-3.6
New Point	-20	-20	-300	91	91	-3.6	-3.6
Good							
Lynnhaven	130	78	330	230	230	23	3.6
Hampton Bar	18	-20	-300	430	91	3.6	-3.6
Polluted							
Little Creek	5,400	230	-3,000	930	91	930	-36
Elizabeth River	1,300	790	-3,000	24,000	1,500	2,400	930
Samples Numbers 1554-N - 1571 inclusive were collected on February 11, 1959.							
Excellent							
Sting Ray Point	-20	-20	-3,000	-36	-36	-3.6	-3.6
New Point	-20	-20	-3,000	-36	-36	-3.6	-3.6
Good							
Lynnhaven	140	45	600	150	91	21	-3.6
Hampton Bar	78	78	-3,000	91	36	-3.6	-3.6
Polluted							
Little Creek	490	330	3,900	1,500	430	240	240
Elizabeth River	16,000	490	1,900	24,000	2,100	1,100	240
Sample Numbers 1687-N - 1704 inclusive were collected on March 9, 1959							
Excellent							
Sting Ray Point	170	-20	-300	430	-36	-3.6	-3.6
New Point	20	-20	-300	-36	-36	-3.6	-3.6
Good							
Lynnhaven	490	170	540	430	230	9.1	9.1
Hampton Bar	9,200	490	730	2,400	430	7.3	3.6
Polluted							
Little Creek	240,000+	35,000	39,000	24,000	24,000	46,000	46,000
Elizabeth River	92,000	5,400	3,800	11,000	430	240,000	4,600
The above sample Numbers 2007-N to 2024-N inclusive were collected and examined on April 29, 1959.							

NOTE - means less than

TABLE A (MR. HOPE AND MR. WILEY)
RAW DATA

	Shell stock			Mud		Sea water	
	Coliform MPN	E. coli MPN	SPC	Coliform MPN	E. coli MPN	Coliform MPN	E. coli MPN
Excellent							
Sting Ray Point	340	-20	500	-3.6	-3.6	3.6	-3.6
New Point	-20	-20	400	-3.6	-3.6	-3.6	-3.6
Good							
Hampton Bar	330	45	900	91	-36	36	-36
Lynnhaven	9,200	950	2,900	91	-36	14	7.2
Polluted							
Elizabeth River	5,400	490	44,000	930	-36	1,100	91
Little Creek	20	-20	-3,000	2,400	93	24,000	4,000
Sample Numbers 2110-N - 2115-N inclusive collected May 18, 1959. Sample Numbers 2116-8-N - 2107-9-N collected May 18, 1959. Sample numbers 2101-2106-N inclusive collected May 18, 1959.							
Excellent							
Sting Ray Point	330	18	620	230	-36	-3.6	-3.6
New Point	45	-20	2,300	230	-36	-3.6	-3.6
Good							
Hampton Bar	3,500	130	470	150	-36	9.1	3.6
Lynnhaven	330	20	400	430	73	43	-3.6
Polluted							
Elizabeth River	28,000	5,400	4,600	2,400	430	46,000	46,000
Little Creek	9,200	790	4,200	46,00	24,000	1,100	93
Sample Numbers 2333-2346-N inclusive; 2335-8 and 2947; 2350, 2341, 2344 2348, 2339, 2342 2349, 2340, 2343-N collected June 22, 1959.							
Excellent							
Sting Ray Point	170	45	330	2,400	-36	3.6	-3.6
New Point	130	-20	-300	43	-36	-3.6	-3.6
Good							
Hampton Bar	45	45	-3,000	23	23	-3.6	-3.6
Lynnhaven	18	-20	270	23	-3.6	9.1	3.6
Polluted							
Elizabeth River	16,000	3,500	5,100	11,000	2,400	2,400	230
Little Creek	24,000	3,500	3,700	24,000	1,100	24,000	1,500
Sample Numbers 2366, 2388, 2382, 2377, 2389, 2383, 2390, 2384, 2364 2386, 2380 2365, 2378, 2387, 2381, 2363, 2385 2379 collected on June 29, 1959.							
Excellent							
New Point	45	45	350	-36	-36	150	3.6
Sting Ray Point	790	130	3,700	1,100	Lost	460	43
Good							
Hampton Flats	230	45	1,600	2,400	930	-3.6	-3.6
Lynnhaven	790	330	480	930	430	43	21

TABLE A (MR. HOPE AND MR. WILEY)
RAW DATA

	Shell stock			Mud		Sea water	
	Coliform MPN	E. coli MPN	SPC	Coliform MPN	E. coli MPN	Coliform MPN	E. coli MPN
Polluted							
Elizabeth River	35,000	9,200	3,700	4,600	1,500	24,000	24,000
Little Creek	35,000	2,200	9,500	24,000	2,400	4,600	1,100
Samples Number 47-64 inclusive collected July 14, 1959.							
Excellent							
New Point	330	-20	LA	230	-36	23	-3.6
Sting Ray Point	2,400	490	LA	230	-36	9.1	-3.6
Good							
Hampton Flats	490	20	LA	430	91	9.1	-3.6
Lynnhaven	460	20	LA	230	36	-3.6	-3.6
Polluted							
Elizabeth River	92,000	5,400	LA	2,400	2,400	4,600	4,600
Little Creek	5,400	490	LA	930	91	1,100	43
Sample Numbers 101-N - 118-N collected July 22, 1959.							
Excellent							
New Point	20	20	400	36	-36	-3.6	-3.6
Sting Ray Point	130	130	-3,000	-36	-36	-3.6	-3.6
Good							
Hampton Flats	3,500	490	-3,000	360	-360	-3.6	-3.6
Lynnhaven	230	78	3,000	930	30	-3.6	-3.6
Polluted							
Elizabeth River	54,000	24,000	14,000	4,300	4,300	24,000	11,000
Little Creek	24,000	1,300	-3,000	24,000	9,300	4,600	200
Sample Numbers 202-N - 219-N collected August 18, 1959.							
Excellent							
New Point	78	20	-300	36	-36	-3.6	-3.6
Sting Ray Point	790	20	650	4,600	73	3.6	-3.6
Good							
Hampton Flats	170	45	17,000	150	36	3.6	3.6
Lynnhaven	3,500	490	630	36	-36	23	3.6
Polluted							
Elizabeth River	5,400	1,300	5,700	24,000	350	2,400	460
Little Creek	5,400	490	4,300	24,000	75	2,400	93
Samples Number 310-N - 327-N collected August 26, 1959.							

TABLE A (MR. HOPE AND MR. WILEY)
RAW DATA

	Shell stock			Mud		Sea water	
	Coliform MPN	E. coli MPN	SPC	Coliform MPN	E. coli MPN	Coliform MPN	E. coli MPN
Excellent							
New Point	230	-20	-300	91	-36	-3.6	-3.6
Sting Ray Point	170	20	-300	36	-36	-3.6	-3.6
Good							
Hampton Flats	5,400	170	410	2,400	-36	9.1	3.6
Lynnhaven	78	-20	-300	150	36	3.6	3.6
Polluted							
Elizabeth River	1,100	490	-3,000	4,600	2,400	1,100	460
Little Creek	2,400	2,400	-3,000	46,000	46,000	240	240
The above samples 407-N - 424-N were collected September 15, 1959.							
Excellent							
New Point	130	-20	-300	210	73	-3.6	-3.6
Sting Ray Point	170	110	230	91	-36	3.6	-3.6
Good							
Hampton Flats	110	78	-3,000	2,400	430	3.6	3.6
Lynnhaven	2,400	45	870	150	93	93	93
Polluted							
Elizabeth River	54,000	4,300	16,000	4,600	11,000	24,000	4,600
Little Creek	5,400	480	3,500	240,000+	46,000	240,000	110,000
The above samples 603-N - 620-N were collected October 19, 1959.							
Excellent							
New Point	330	-20	-300	-36	-36	-3.6	-3.6
Sting Ray Point	330	-20	540	36	36	-3.6	-3.6
Good							
Hampton Flats	3,500	1,100	5,000	2,400	430	93	43
Lynnhaven	2,200	700	4,900	24,000+	24,000+	240	43
Polluted							
Elizabeth River	240,000+	35,000	210,000est	24,000	24,000	11,000	11,000
Little Creek	35,000	3,500	12,000	240,000+	240,000+	11,000	4,600
The above samples 675-N - 692-N were collected October 28, 1959.							

TABLE B (MR. HOPE AND MR. WILEY)
SPECIFIC GRAVITY, SALIMETER AND TEMPERATURE READING

	LITTLE CREEK		ELIZABETH RIVER		LYNNHAVEN RIVER		HAMPTON ROADS		NEW POINT		STING RAY POINT	
DATE	SP.GR.	TEMP.*	SP. GR.	TEMP.*	SP. GR.	TEMP.*	SALIMETER	TEMP.*	SALIMETER	TEMP.*	SALIMETER	TEMP*
9-15-59	1.016	27	1.014	26	1.020	24	- - - -	- -	- - - -	- -	- - - -	- -
10-19-59	1.015	19	1.014	20	- - -	- -	- - - -	19	- - - -	19	- - - -	17
10-28-59	1.010	14	- - -	- -	1.016	15	- - - -	17	- - - -	17	- - - -	16.5
6-29-59	1.014	31	1.012	29	1.014	31	- - - -	- -	- - - -	- -	- - - -	- -
6-22-59	1.014	29	1.012	25	1.018	26	- - - -	24	- - - -	24	- - - -	24
4-29-59	1.014	17	1.012	18	1.014	16½	- - - -	16	- - - -	15	- - - -	- -
7-14-59	1.004	27	1.006	25	1.014	27	- - - -	- -	- - - -	- -	- - - -	- -
7-22-59	1.012	28	1.011	28	- - -	- -	- - - -	- -	- - - -	- -	- - - -	- -
8-18-59	1.014	23	1.014	29	1.016	29	- - - -	27.5	- - - -	26	- - - -	27
8-21-59	- - -	- -	- - -	- -	1.016	29½	- - - -	- -	- - - -	- -	- - - -	- -
8-26-59	1.014	30	1.014	30	- - -	- -	- - - -	29	- - - -	29	- - - -	28
5-18-59	1.012	19	1.014	19	1.013	19	- - - -	19	- - - -	- -	- - - -	- -
2-11-59	1.016	9	1.016	8	1.018	7	7.0%	6.5	7.0%	5	6.0%	6
1-20-59	- - -	- -	- - -	- -	- - -	- -	7.0%	3	- - - -	- -	- - - -	- -
11-17-59	- - -	- -	- - -	- -	- - -	- -	- - - -	- -	7.0%	- -	6.5%	- -
11-10-59	- - -	- -	1.014	15	1.016	14	7.0%	13	- - - -	- -	6.7%	13
10-23-59	- - -	- -	- - -	- -	- - -	- -	- - - -	- -	5.0%	17	5.5%	16
10-27-59	- - -	- -	- - -	- -	- - -	- -	6.0%	17	5.5%	16	5.0%	14.5
10-29-59	1.016	17	1.015	17	- - -	- -	6.5%	15	6.0%	15	5.0%	14.5
3-9-59	1.015	9	1.014	9	1.016	10	7.0%	8	6.0%	7	6.0%	8
1-26-59	- - -	- -	- - -	- -	- - -	- -	7.0%	5	7.5%	2.5	7.0%	4

APPENDIX C AREA POLLUTION SOURCES RELATED TO STUDY

	Marinas Boat Basin or Railway	Animal	Raw Sewage	Municipal Terminal	S.T.P.*	P.S.*	No Facilities	Privy	Septic Tank
New Point	1	3	- - -	- - -	- -	- -	- -	- -	- -
Sting Ray Point	12	3	- - -	- - -	- -	- -	1	2	5
Hampton Flats	4	- -	1	1	1	4	- -	- -	- -
Lynnhaven	3	4	- - -	- - -	13	- -	- -	- -	- -
Elizabeth River	4	- -	4	- - -	13	- -	- -	- -	- -
Little Creek	6	- -	1	- - -	6	- -	- -	- -	- -

NOTE S.T.P.= Sewage Treatment Plant
P.S. = Pumping Station

APPENDIX I

THE SIGNIFICANCE OF EC POSITIVE ORGANISMS IN GULF SHELLFISH GROWING WATERS

By

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and

C. B. Kelly, Chief
Shellfish Sanitation Laboratory

Arthur N. Beck, G. Russell Wright,
J. L. Crockett, Jr., and William S. Phillips

In the limited time available, it is not possible to describe in adequate detail the data accumulated in our 1960-1961 study of the Mobile Bay and oyster bearing areas. This investigation involved the weekly testing of 43 stations for one year or 2,392 coliform determinations by the three tube test. Additionally, all positive lactose tubes were reinoculated into EC media and incubated in a water bath at $44.5^{\circ} \pm 0.2^{\circ}$ for 24-48 hours. For some time, after incubation, all EC tubes positive or negative were streaked to eosin methylene blue plates. After incubation, one colony conforming to the accepted morphology of E. coli, or if no such were present, a colony which came closest to being typical, was picked and inoculated into a lactose tube. From this tube the EC test was repeated along with IMViC determination. It soon became apparent that plating of EC negative tubes was not fruitful so the routine was altered, plating only positive EC specimens. In all some 10,000 IMViC and repeat EC determinations were performed. No tests were performed on oysters but this is now under study.

Printed material has been distributed to you for study at your leisure and includes, again, only a partial presentation of the data. It is sufficient, however, to indicate the significance of EC positive organisms in Gulf shellfish growing waters.

The Mobile Bay area is roughly some 30 to 35 miles long and approximately 25 miles at its widest point. The upper part of the bay shows extreme pollution which has no effect on the Cedar Point growing areas under normal conditions. During the rainy season this immunity to contamination may abruptly change. It may be added here that last February and March Alabama experienced the worst floods in recorded history.

Figure 1 shows a general picture of the station locations as well as the normal current flow and flood pattern. A few of the upper stations, including the Alabama and Tombigbee stations, are not shown in this chart. By studying the various stations it was apparent that some areas could be grouped rather than considered independently. Alphabetically, therefore, grouped or single stations were, in their descending order down the bay, starting with the Alabama and Tombigbee Rivers, designated as Group A, B, etc. In general we shall only discuss Group H, J, L and M. The station or stations represented by this grouping may be identified in the table showing probability percentiles.

Figure 2, Station 31 adequately shows the high pollution usually present. The overall trends suggest diluting out of EC gas positive organisms derived from the fresh sewage discharges by fresh water during periods of increased river flow. This is apparent beginning in late October, with progressively increasing separation of the coliform and EC lines throughout the flood stage which persisted util late May. When normal river flows reoccurred, there was a return to a similar pattern of the coliform and EC MPN's.

Station 48 (Figure 3) is included because it represents an area affected by run-off which reached a peak towards the end of March. The rythmical pattern of coliform and EC MPN spearations were disrupted when salinity decreased. The marked increase in run-off resulted in a doubled coliform count but the EC MPN was only slightly changed. This continues until recovery of normal salinities is apparent.

Some 35 miles down the bay from Station 31 we have used Station 87 (Figure 4) as an index area. During normal salinity content in the bay the coliform count, though fluctuating, reaches sizeable peaks while the EC count remains less than three. Around the 20th of March, with falling salinity, pollution occurred and there is an immediate rise in EC MPN's. The pattern of recovery shows gradual return to the usual pattern of coliform-EC relationships.

Station 119 (Figure 5) is directly over the Cedar Point oyster area and I think illustrates the response of the EC test to pollution. The concurrent drop in salinity and rise in EC MPN's is dramatically evident.

The histogram (Figure 6) summarizes in percentage the confirmed EC positive as compared to coliform positive tubes and shows clearly the response of EC media to pollution as compared to the accepted coliform test. You will note that during period 1 at Group G (Station 20) immediately below the discharge of fresh sewage, the EC test was 83 percent positive compared to the coliform test and there was a gradual declineuntil Group M (oyster bed area) was reached where only 9.8 percent of the coliform positive tubes were EC positive. During period 2, flood stage, no change in percent EC positiveness was apparent in area G. Some dilution by river water caused the reduction in percent EC positives at groups H and J. The most striking difference occurred in the areas immediately above and over the oyster beds. Sharp increases in percent EC gas positives occurred. These can be explained by the influx of fresh water that received pollution in the Mobile area and, as shown in Figure 5, reached the oyster beds during the flood stage. The fact that travel time was considerably less during the flood stage with the associated reduction in die off rate might also be a factor which influenced the increases in percent EC gas positiveness. It is also significant that when river discharges returned to normal low rates (recovery period), the progressive decrease in percent EC positiveness, associated with distance from pollution, was demonstrated.

Table I shows these same grouped stations as coliform and EC percentile probabilities. Again, as we descend the bay, improvement is noted over the oyster beds (Group M) the 50 and 90 percentiles fall well within accepted norms during the normal and recovery periods but completely outside the range during the flood stage.

Now, I want to throw away the manuscript for one minute and say something about the technique and performance of the E. C. test. From 8,400 positive E. C. tests incubated for 24 to 48 hours we recovered the following: 24 hour period 88.4 percent types I and II E. coli strains, 7.9 percent irregular VI's and 1.3 percent as other. By contrast, incubation for an additional 24 hours resulted in the recovery of only 0.2 percent more E. coli but an additional two percent were irregular VI's and others. Should this trend continue it seems to me that we are lowering the specificity of the test without increasing the sensitivity by the additional 24 hour incubation.

During this conference there has been some discussion as to the temperature limits required in the running of the E. C. test. We recently subjected some 600 strains of types I and II E. coli and 161 isolates of A. aerogenes to the E. C. test run at 44.5 and 45 C. Raising the temperature one half degree eliminated 60 percent of the aerogenes isolates while less than one percent of the E. coli strains were lost. These results will probably require further confirmation and verification before any changes in the temperature level should be considered.

Finally, once you have determined your own confidence and reactivity levels of the E. C. test by the IMViC testing procedure only occasional IMViC testing seems necessary.

Summary

1. A Sanitary Survey of Mobile Bay was conducted September 15, 1960 - September 1961. Samples were collected at weekly intervals and were tested for coliform organisms and fecal coliform organisms, with classification of the coliforms by IMViC test.

2. Conditions of low and high runoff were encountered with the consequent change in travel of pollution down the bay. During the Spring of 1961, the worst floods in recorded history were experienced.

3. During periods of low flow, coliform densities in the lower portion of the bay were sufficiently low to justify allowing the use of the area for the cultivation and harvesting of oysters.

A more significant association with pollution conditions was revealed by the EC test since the proportion of coliforms that responded positively to the test decreased with remoteness from pollution.

4. During flood stages, decrease in travel time resulted in a reduction in die off of coliform organisms. Although there occurred a simultaneous increase in coliform organisms a dramatic increase in percentage EC gas positiveness occurred, again indicating the advantage of the fecal coliforms as an index of fecal pollution. Over the oyster beds the percent of EC gas positives returned to a low level during the recovery period while fluctuations in coliform densities occurred.

MPN and salinity charts represent a two item moving average.

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Financial assistance for this study was made possible through the efforts of Dr. D. G. Gill, State Health Officer.

****

This has been a joint research program in which the following participated:

State Health Department, Montgomery, Alabama:

Bureau of Sanitation, Arthur N. Beck, Chief, and staff
Bureau of Laboratories, Thomas S. Hosty, Chief, and staff
C. B. Kelly, Chief, Shellfish Sanitation Laboratory,
Public Health Service

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Specimens were collected at weekly intervals beginning on October 1, 1960. In some instances other stations were picked up at a later date. The MPN and Salinity charts represent a moving average x 2.

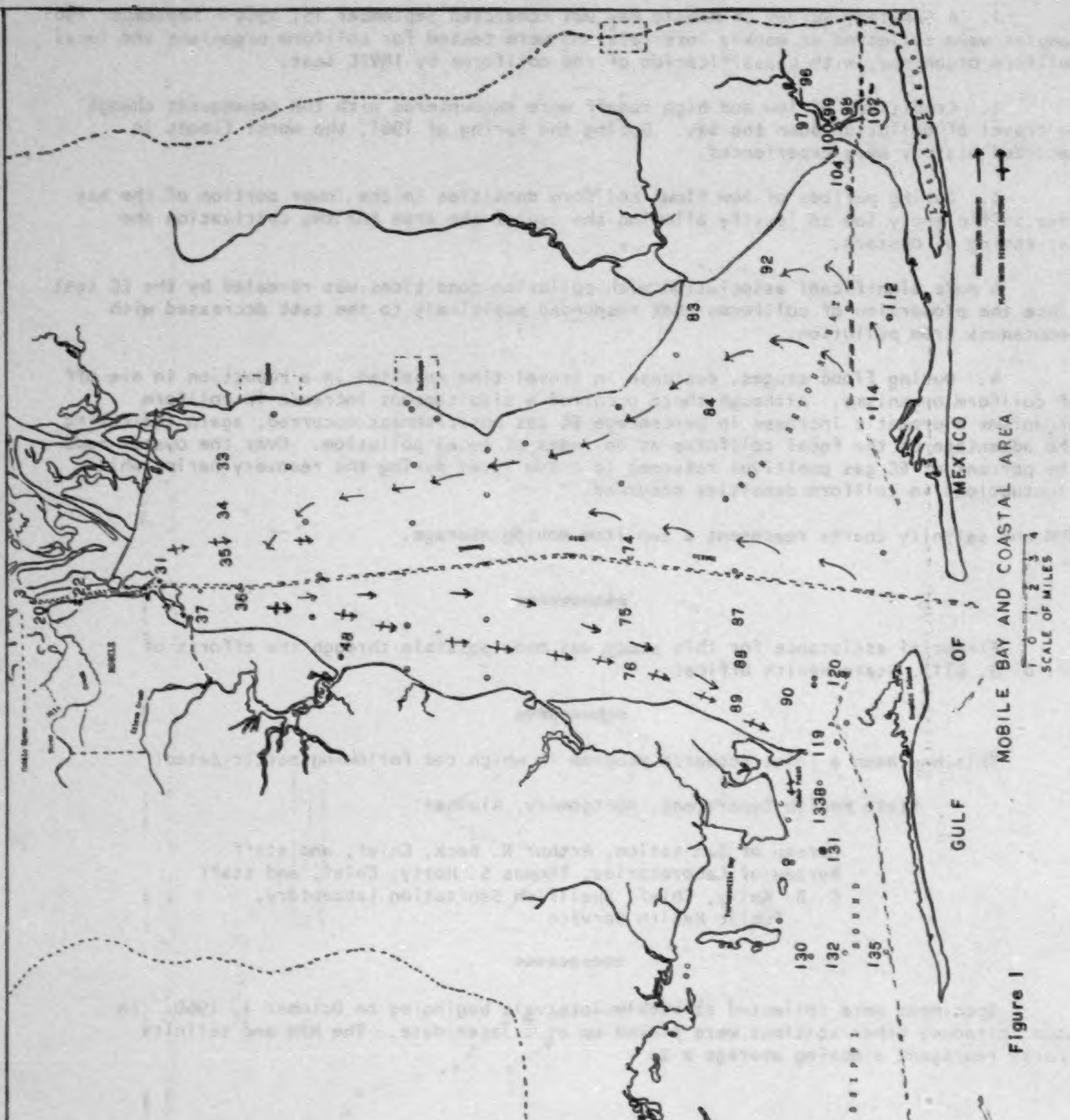
GULF OF MEXICO
MOBILE BAY AND COASTAL AREA
SCALE OF MILES
0 1 2 3
MOBILE
31
33
34
35
36
37
74
75
76
83
84
87
88
89
90
92
96
97
98
99
102
104
112
114
119
120
130
131
132
133
135
8
20
21
22
SOUND

Figure 1

STATION 31
COLIFORM and EC MPN'S with SALINITY CHANGES
SEPTEMBER 1960 to SEPTEMBER 1961

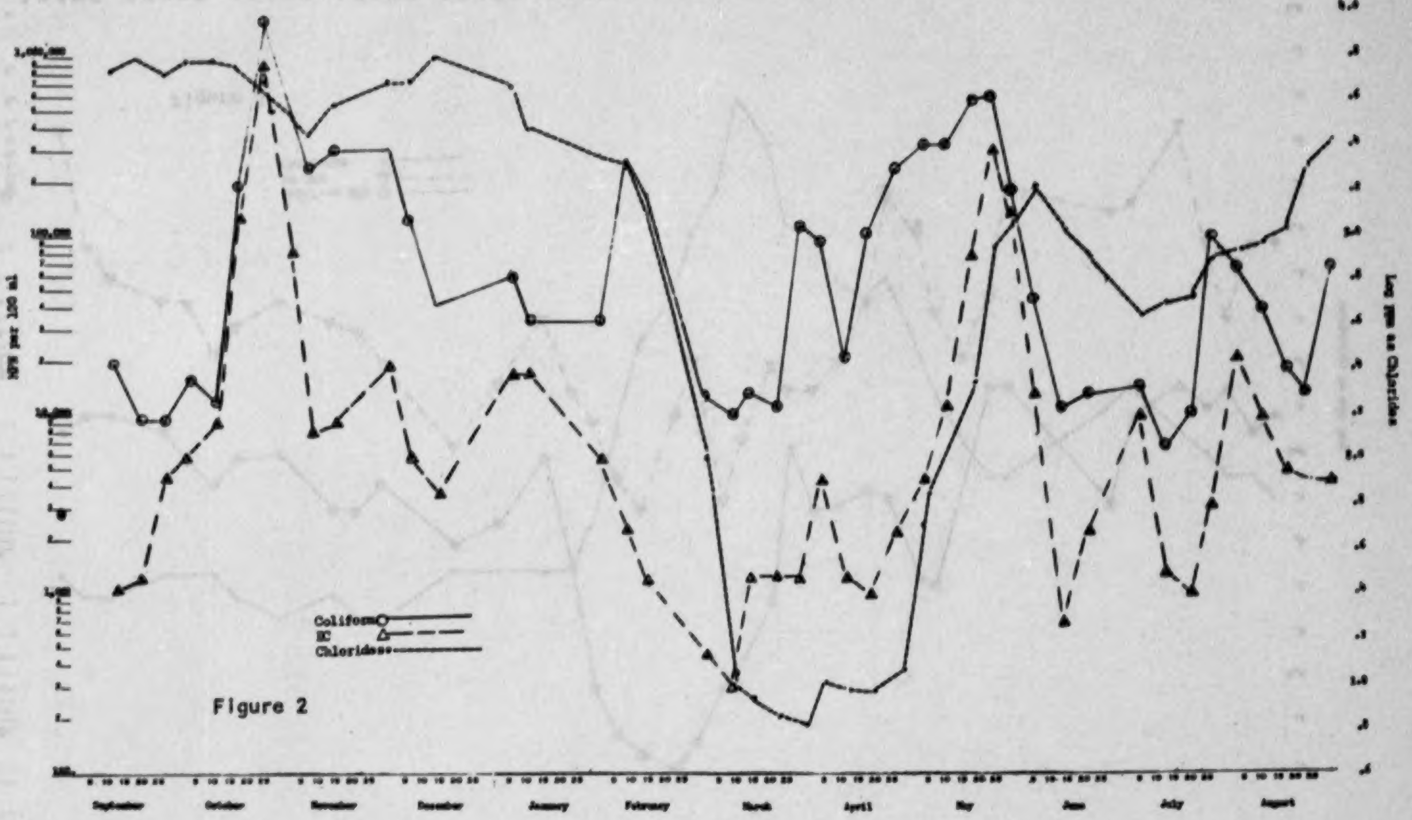

Figure 2

STATION 48
COLIFORM and EC MPN'S with SALINITY CHANGES
SEPTEMBER 1960 to SEPTEMBER 1961

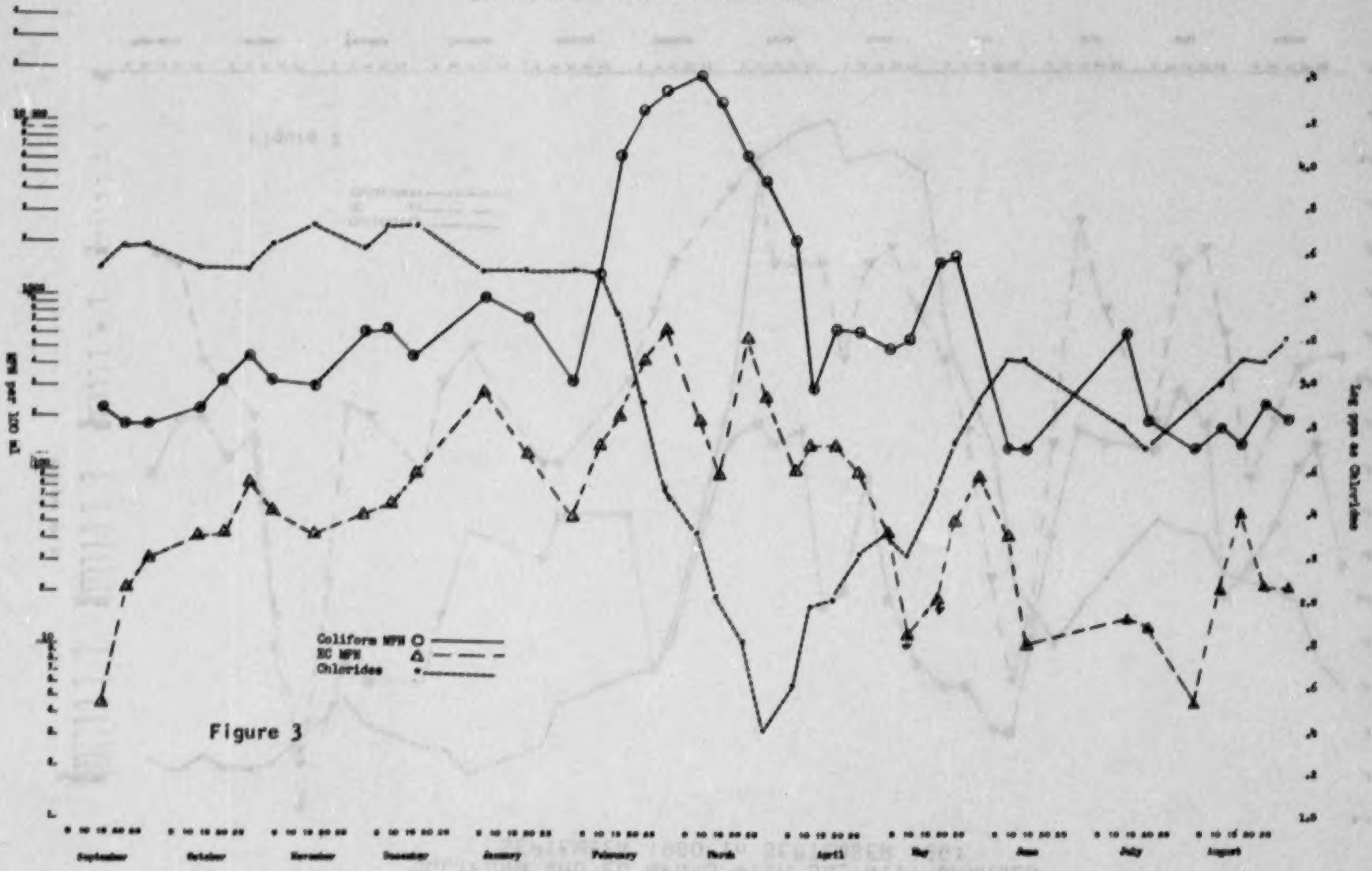

Figure 3

STATION 87
COLIFORM and EC MPN'S with SALINITY CHANGES
SEPTEMBER 1960 to SEPTEMBER 1961

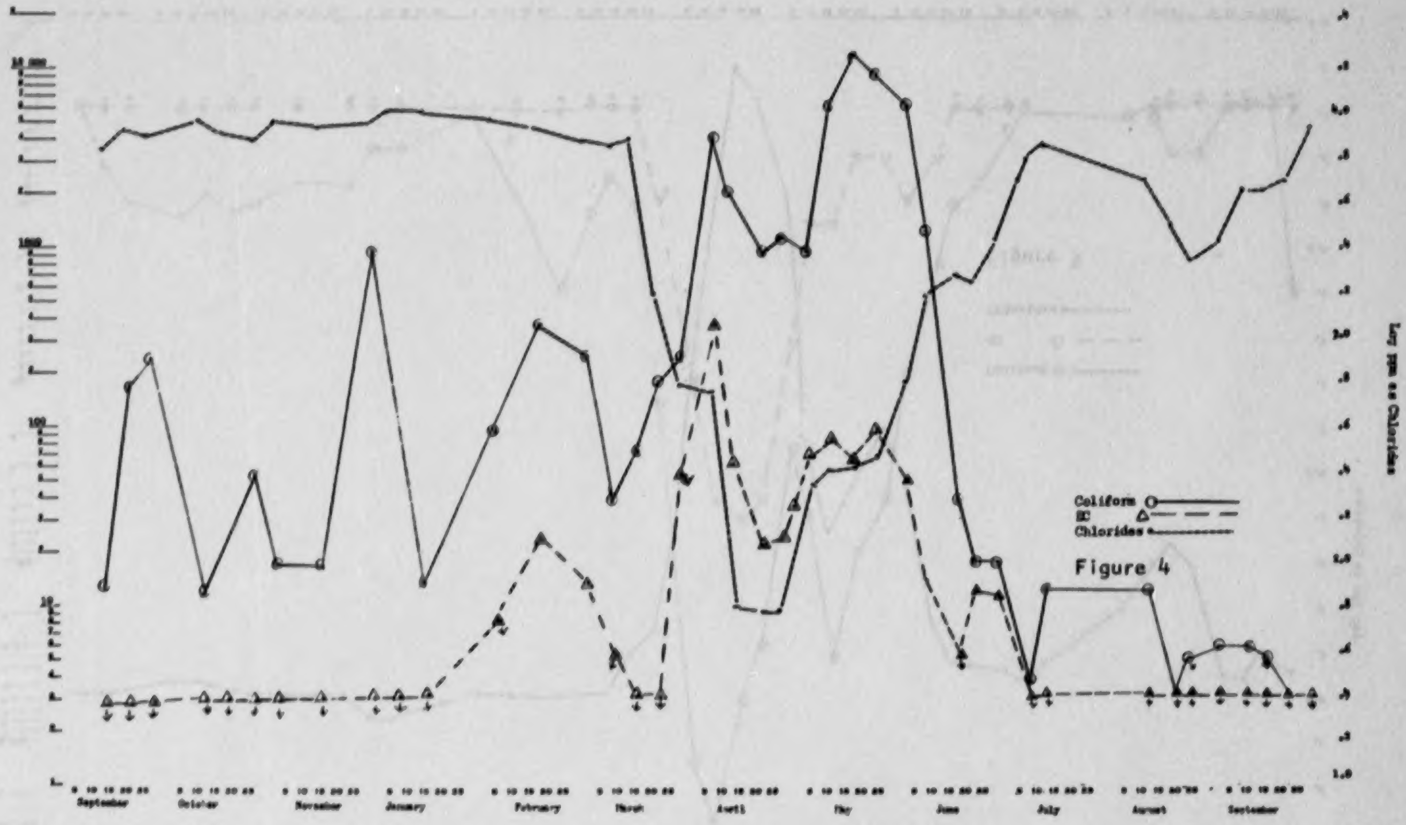

Figure 4

STATION 119
COLIFORM and EC MPN'S with SALINITY CHANGES
SEPTEMBER 1960 to SEPTEMBER 1961

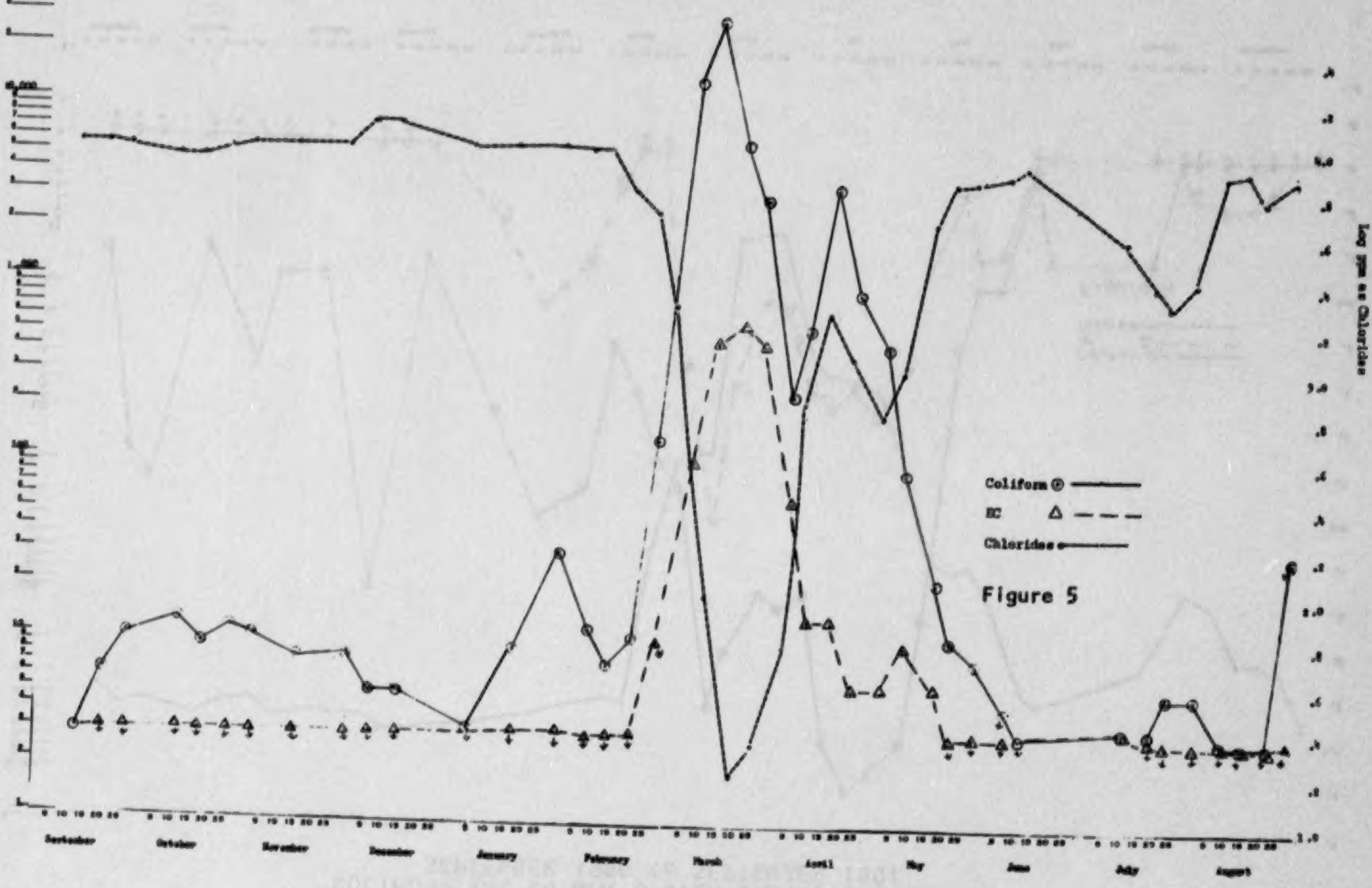

Figure 5

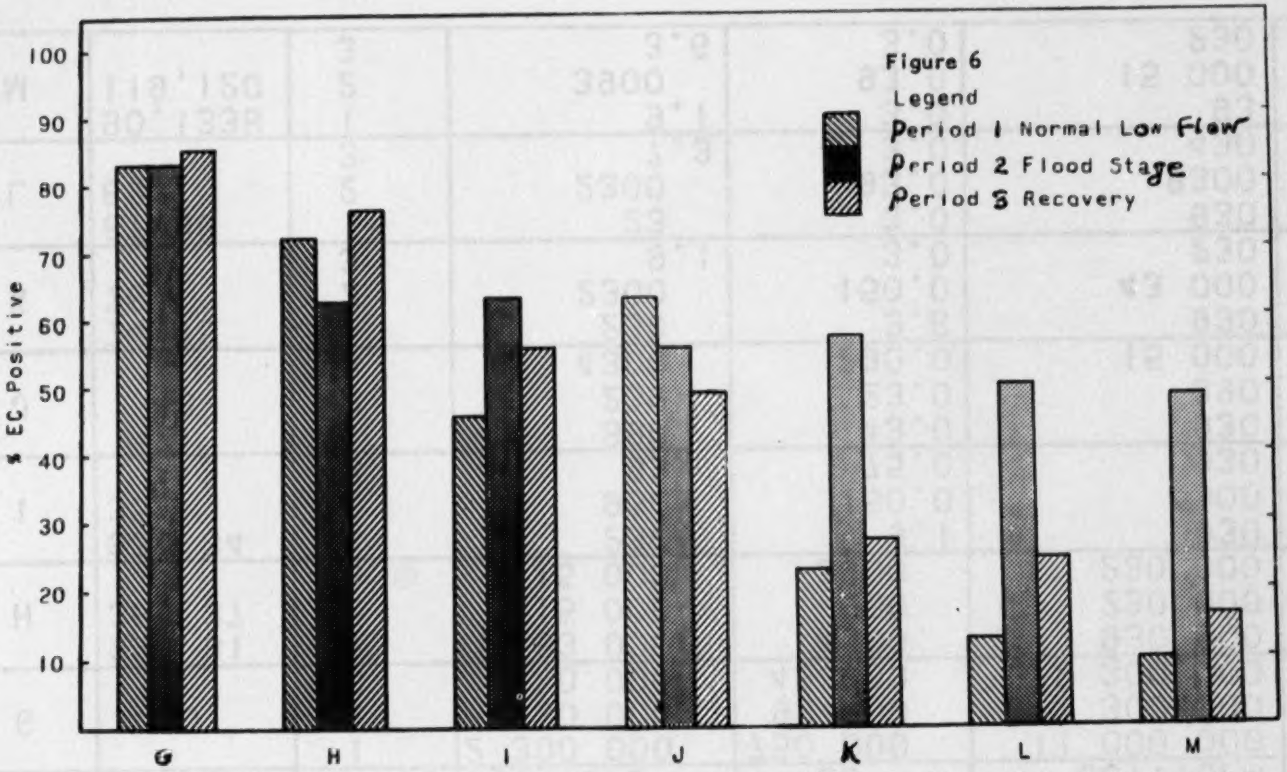

Figure 6

Relation of Confirmed EC Positive Tubes to Coliform Positive Tubes by Period

TABLE I

Coliform and EC Percentile Probabilities by Station and Period

Group	Station	Period	50%		90%	
			Coliform	EC	Coliform	EC
G	20	1	2 300 000	750 000	11 000 000	4 300 000
		2	930 000	93 000	2 300 000	430 000
		3	750 000	43 000	2 300 000	750 000
H	22, 31	1	93 000	4300	930 000	23 000
	36, 37	2	15 000	930	230 000	4300
		3	15 000	3900	230 000	75 000
I	33, 34	1	230	9.1	930	230
	35	2	930	150.0	4300	750
		3	93	75.0	930	93
J	48	1	390	43.0	930	150
		2	230	23.0	390	120
		3	4300	230.0	15 000	430
K	74,75	1	230	3.6	930	23
	76	2	2300	150.0	43 000	430
		3	9.1	3.0	230	21
L	87,88	1	23	3.0	930	9.1
	89	2	2300	93.0	9300	430
		3	7.3	3.0	430	9.1
M	90,133B	1	9.1	3.0	93	3.6
	119,120	2	3900	93.0	15 000	430
		3	3.6	3.0	230	7.3

APPENDIX J

AN EVALUATION OF THE E.C. (44.5°C.) CONFIRMATION TEST FOR THE ESTIMATION OF ESCHERICHIA COLI DENSITIES IN SEA WATER AND SHELLFISH

By

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Section I. Introduction

Since 1953 there has been a considerable degree of interest in Canada and the United States in the development of an effective, relatively simple test for the estimation of Escherichia coli densities in shellfish and shellfish growing waters. Bacteriological control, based on the determination of all members of the coliform group capable of lactose fermentation at 35.5°C., has proven reasonably satisfactory; it has been found difficult, however, to relate coliform densities to positive public health hazard in some shellfish growing areas, particularly during periods of rainfall-induced run-off. The presence of E. coli has generally been regarded as positive proof of fecal contamination. The routine application of a test for this organism in shellfish and sea water, however, has awaited the development of an accurate, simple procedure which does not involve confirmation through pure culture isolation. Selective examination for E. coli by means of elevated incubation temperatures (43° - 46°C.) has received much study since the work of Eijkman in 1904. Presumptive test for E. coli using an incubation temperature circa 44°C., are widely used, particularly in Europe, for the determination of bacteriological quality in water and shellfish. A "subculture", or "confirmation" test for lactose fermentation at 44° - 44.5°C., appears to have considerable advantage over a presumptive test. Widespread interest and study in the United States and Canada have culminated in the development of the E.C. Test, and its adoption as the sole coliform criterion of pollution and mishandling of shucked market oysters. Modifications of the present test have been used in every shellfish growing area study conducted by the Laboratory of Hygiene since 1953; the data obtained have been useful in establishing pollution levels and closure lines.

The E.C. Confirmation Test is used as an adjunct to the Standard Methods Test for members of the coliform group. All positive presumptive lactose broth cultures are subcultured to tubes of E.C. Medium for 24 - hour incubation at 44.5°C. Gas production has been considered to indicate the presence of E. coli type I, although it is known that occasional strains of other coliform types are also E.C. gas positive at 44.5°C. The present report concerns an evaluation of the E.C. Test, and of the degree of interference caused by "aberrent" Aerobacter and intermediate coliform strains, conducted during the summer of 1959 in conjunction with shellfish growing area surveys in Quebec and Prince Edward Island.

A. Summary of Project

A total of 12 shellfish growing areas were studied - six on the north shore of the estuary of the St. Lawrence River in the Providence of Quebec (May 26 to July 20, 1959), and six in the Province of Prince Edward Island (August 3 to October 30, 1959). A total of 3530 sea water samples and 85 shellfish specimens taken periodically from fixed sampling stations in the study areas were subjected to the APHA Standard (Most Probable Number) Test for coliform bacteria and the E.C. (44.5°C. gas positive) Confirmation Test. The coliform organisms responsible for gas positivity in 2765 E.C. cultures from 973 of these samples were isolated and classified according to their IMViC reactions.

B. Conclusions

E. coli and its indol-negative form, Irregular variety II, have generally been regarded as of definitely "fecal" origin. Since the presence of these coliform biotypes could be demonstrated in 95.3 per cent of 1,499 EC gas positive cultures from Prince Edward Island sea water and shellfish, it may be inferred that the EC Confirmation Test is a reasonably precise procedure for the identification of "fecal" coliform organisms. Data obtained from the studies in the Province of Quebec have shown, however, that the relationship between E.C. gas positivity and the presence of "fecal" coliform strains is not always so precise; only 84 per cent of 1,266 E.C. gas positive cultures from Quebec sea water and shellfish specimens could be shown to contain "fecal" coliform strains. Moreover, the data demonstrate that the per cent recovery of "fecal" strains was lower in moderately polluted sea water than in sea water subject only to negligible pollution. The few coliform bacteria present in the relatively unpolluted areas were thus predominantly "fecal" in type, while sewage entering the moderately polluted areas was characterized by a wider distribution of coliform biotypes.

Unless all the coliforms in the unpolluted areas can be attributed entirely to direct pollution by sea birds or by land-wash, fecal pollution of these waters must necessarily be from a distant source and one must postulate an extended selective survival for E. coli in sea water to explain the predominance of this type in sea water remote from direct human and animal pollution. This is quite contrary to the consensus of earlier workers who contended that Aerobacter and intermediate coliforms survive longer than E. coli in soil and water. It would appear further study on this point is desirable.

In conclusion, it appears that the E.C. Confirmation Test provides a reasonably efficient, simple method for the estimation of "fecal" coliform densities in sea water and shellfish, but that the precision of the method varies considerably from area to area. While no attempt has been made to evaluate the negative aspect of the Test, this study has shown that under favorable conditions the E.C. Test is reasonably specific for E. coli. The Test is less specific, however, when E. coli strains are a minority group in the total coliform flora. Other E.C. gas positive coliform biotypes, particularly A. aerogenes varieties I and II, are a definite threat to the specificity of the test, and these organisms have been shown to be present in significant numbers in moderately polluted sea water. We regard the E.C. Confirmation Test as a useful, effective method for the determination of E. coli densities, but we do not believe that it should replace the standard coliform test in the bacteriological control of Canadian shellfish growing areas.

Section II. Description of Study Areas

A. QUEBEC AREAS

The Quebec investigation covered a 125-mile sector of the north shore of the St. Lawrence River estuary. This region is heavily wooded and sparsely settled; most of the homes are well back from the River, and a few towns and villages constitute the only significant sources of domestic pollution. There are no sewage treatment plants in the region. For purposes of discussion, the sector was divided into six study areas:

Q-1. Baie Comeau to Pointe Lebel

Two rapidly expanding towns, Baie Comeau (pop. 9,000) and Hauterive (pop. 5,000), contribute domestic sewage directly to the waterfront via municipal sewers. Hospitals in each town discharge wastes to the same sewers, and a large paper mill at Baie Comeau contributes sulfite and other wastes directly to tidewater. The sector covered by sampling stations 1 to 40 inclusive (Fig. 1) may thus be considered to be grossly polluted. The remainder of the study area appears to be free from serious direct pollution; the few homes are well back from the waterfront, and the Manicouagan River does not appear to contribute pollution to the south shore of its estuary.

Q-2. Pointe Lebel to Riviere aux Vases

The villages of Pointe Lebel and Pointe aux Outardes are the only populated areas in the sector covered by sampling stations 1 to 69 inclusive (Fig. 2). There are no municipal sewers and in no instance are homes located within 200 yards of the water. The remainder of the area receives some pollution from scattered homes situated near streams (Riviere a la Truite, Ruisseau Vert and Riviere aux Vases) flowing to the study area. Construction work at a bridge site near the mouth of Riviere a la Truite may have had some influence on the bacteriological data obtained from adjacent sampling stations. On the basis of all available public health engineering information, pollution in the study area was judged to be negligible to slight.

Q-3. Riviere aux Vases to Banc des Canadiens

Forty homes along the waterfront contribute domestic wastes to the sector covered by sampling stations 1 to 19 inclusive (Fig. 3). This sector is also subject to pollution from homes situated along two streams (Riviere aux Vases and Riviere aux Rosiers) which flow into the area.

The sector of the study area covered by sampling stations 53 to 82 inclusive receives pollution from the Betsiamites Indian Reserve (pop. approximately 1,100); sewage from the Reserve flows directly into a small bay near sampling station 64, and ultimately into the Betsiamites River which also receives domestic wastes from homes located along the River upstream from the study area.

The remainder of the area (stations 20 to 52 inclusive) is rocky, heavily forested and very sparsely inhabited, and no significant sources of pollution were noted; it is probable, however, that sewage from the polluted sectors will affect water quality in this portion of the area under certain conditions. In summation, pollution levels in the study area were rated as slight to gross.

Q-4. Banc des Canadiens to Riviere Laval

There are very few sources of human pollution on the immediate shoreline of this area; the few homes are all more than 100 feet from the water. Two streams, Riviere Colombier and Riviere Blanche, contribute some pollution from upstream homes to the sector covered by sampling stations 62 to 67 inclusive (Fig. 4). Heavy concentrations of sea birds were present near stations 1 to 11 inclusive during the study. The pollution potential of this area might be rated as slight to moderate.

Q-5. Riviere Laval to Ste.Anne de Portneuf

Two homes are located on the immediate shoreline near sampling stations 1 to 5 inclusive (Fig. 5). Riviere Jean Raymond may be expected to contribute moderate pollution to the area. Major pollution sources are located along Riviere Sault au Cochon, at Forestville (pop. 3,000), and at pulp-loading wharves near sampling stations 17 and 18. Sewage from 40 homes and a school at the town of Portneuf reaches tidewater either directly or indirectly via a small stream. The shoreline between sampling stations 26 and 42 is bordered by 40 - foot cliffs and is completely uninhabited. It may be concluded that pollution levels in the area range from moderate to slight.

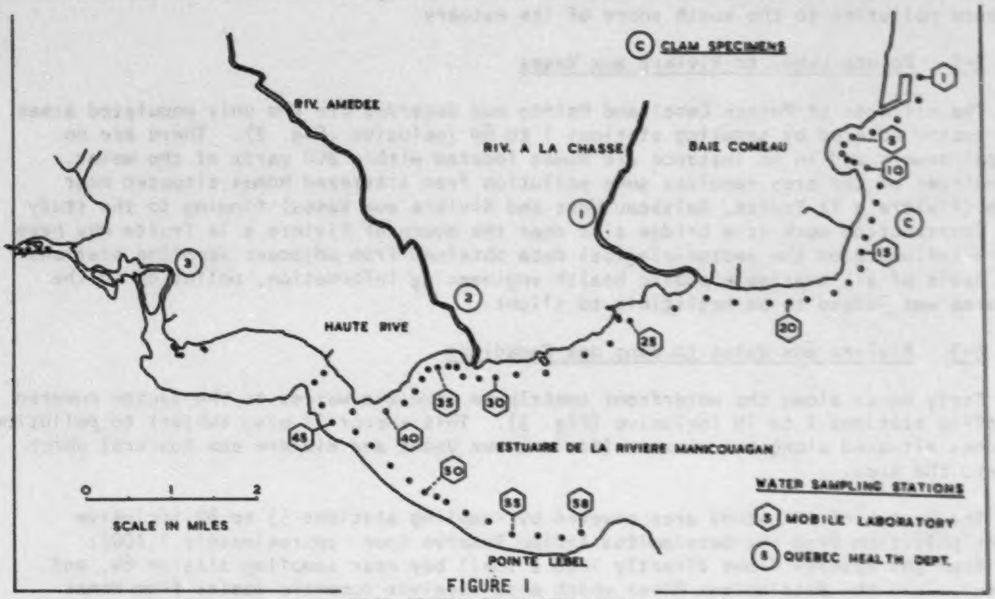
C CLAM SPECIMENS
RIV. AMEDEE
RIV. A LA CHASSE
BAIE COMEAU
HAUTE RIVE
ESTUAIRE DE LA RIVIERE MANICOUAGAN
POINTE LEBEL
0 1 2
SCALE IN MILES
WATER SAMPLING STATIONS
5 MOBILE LABORATORY
5 QUEBEC HEALTH DEPT.
1
5
10
C
15
20
1
2
3
25
30
35
40
45
50
55
58

FIGURE 1
BAIE COMEAU TO POINTE LEBEL

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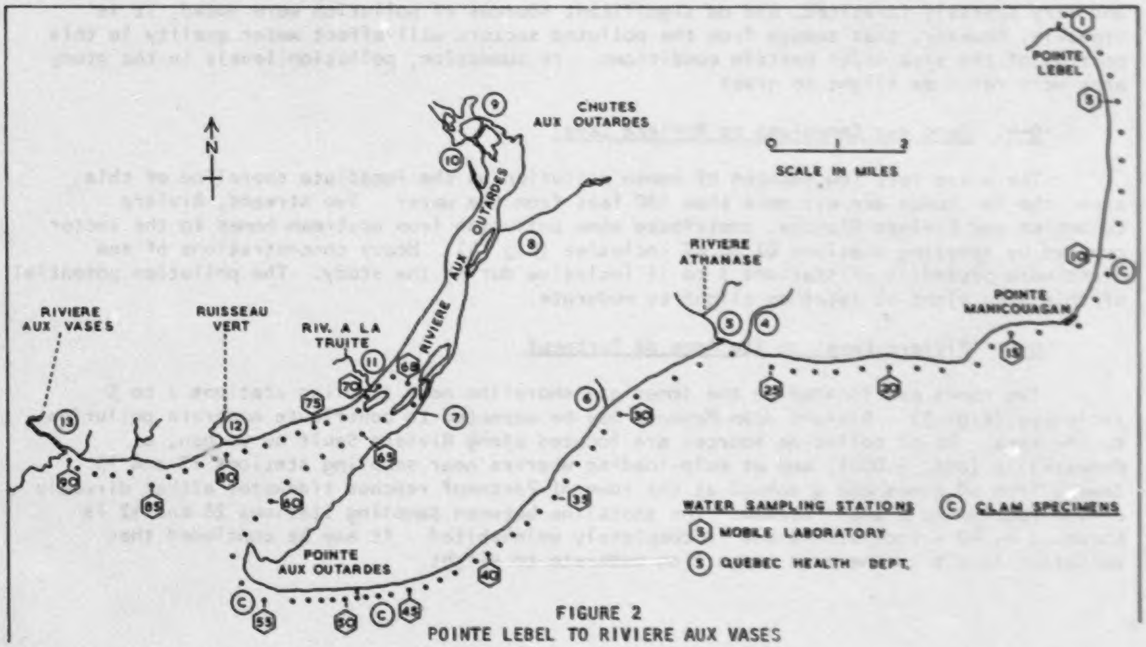

N
CHUTES AUX OUTARDES
0 1 2
SCALE IN MILES
POINTE LEBEL
RIVIERE ATHANASE
POINTE MANICOUAGAN
RIVIERE AUX VASES
RUISSEAU VERT
RIV. A LA TRUITE
RIVIERE AUX OUTARDES
POINTE AUX OUTARDES
WATER SAMPLING STATIONS
C CLAM SPECIMENS
5 MOBILE LABORATORY
5 QUEBEC HEALTH DEPT.
1
5
9
10
8
C
4
5
6
7
11
12
13
15
25
30
35
40
45
50
55
60
65
68
70
75
80
85
90

FIGURE 2
POINTE LEBEL TO RIVIERE AUX VASES

1959 BACTERIOLOGICAL SURVEYS

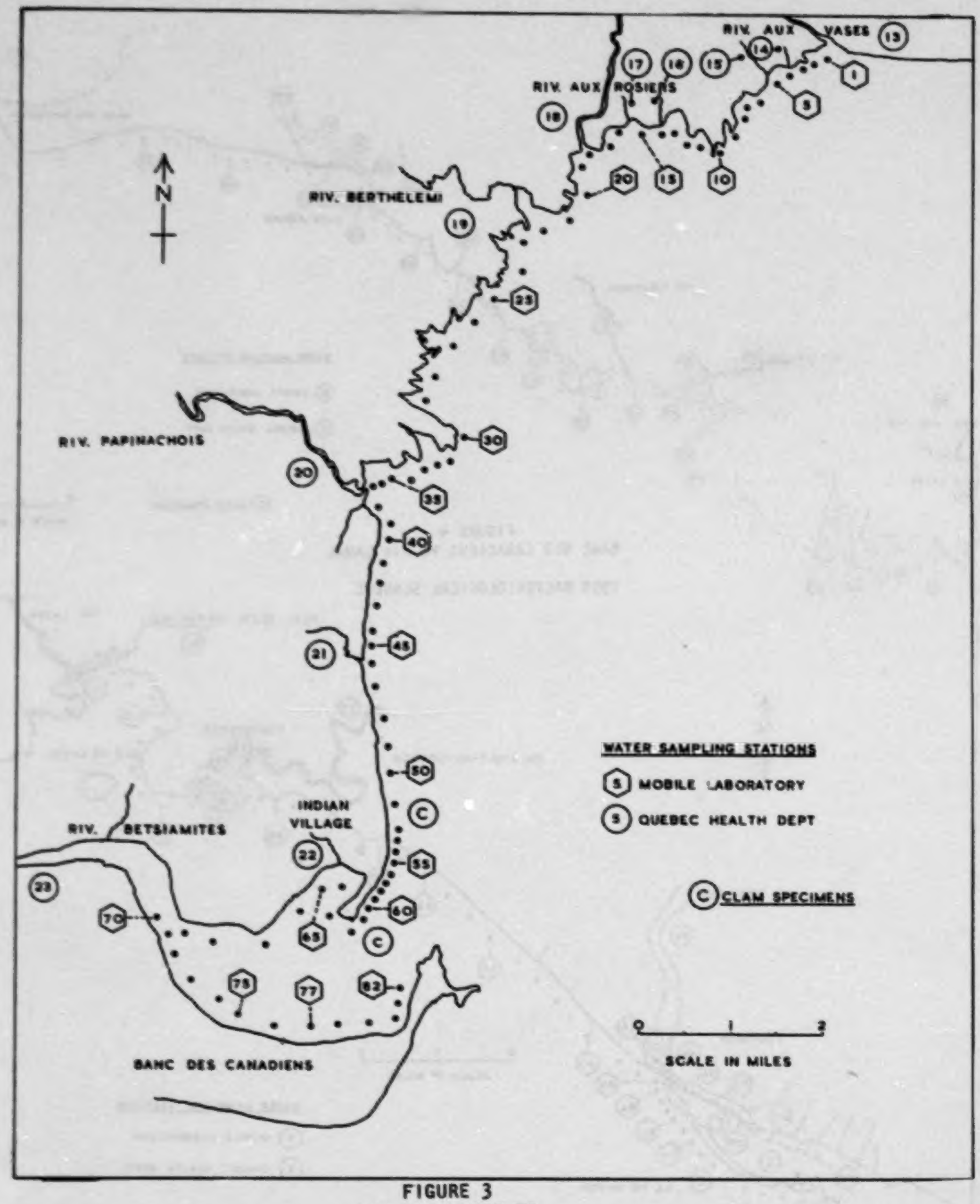

N
RIV. AUX VASES
RIV. AUX ROSIERS
RIV. BERTHELEMI
RIV. PAPINACHOIS
RIV. BETSIAMITES
INDIAN VILLAGE
BANC DES CANADIENS
WATER SAMPLING STATIONS
5 MOBILE LABORATORY
5 QUEBEC HEALTH DEPT
C CLAM SPECIMENS
0 1 2
SCALE IN MILES

FIGURE 3
RIV AUX VASES TO
BANC DES CANADIENS

1959 BACTERIOLOGICAL SURVEYS

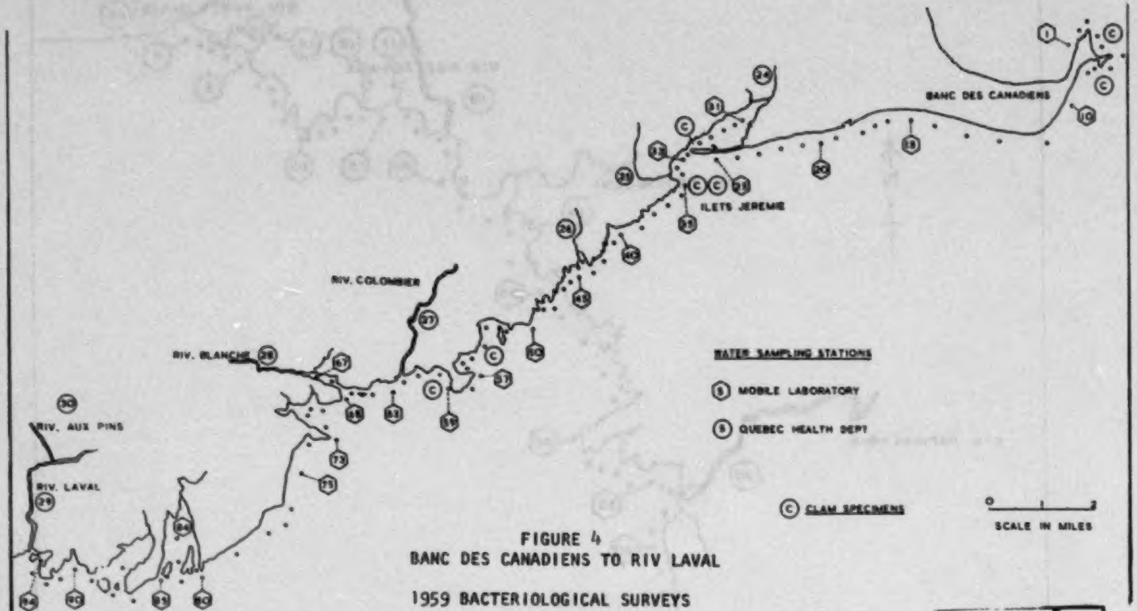

BANC DES CANADIENS
ILETS JEREMIE
RIV. COLOMBIER
RIV. BLANCHE
RIV. AUX PINS
RIV. LAVAL
WATER SAMPLING STATIONS
MOBILE LABORATORY
QUEBEC HEALTH DEPT
CLAM SPECIMENS
0 1 2
SCALE IN MILES

FIGURE 4
BANC DES CANADIENS TO RIV LAVAL

1959 BACTERIOLOGICAL SURVEYS

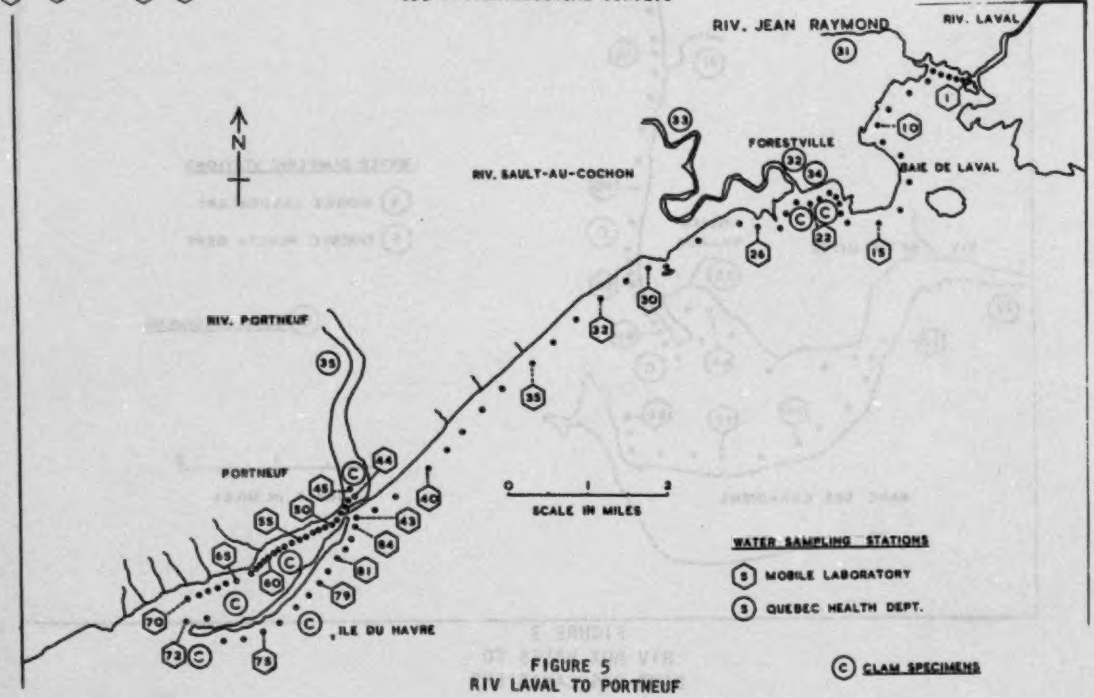

RIV. JEAN RAYMOND
RIV. LAVAL
FORESTVILLE
BAIE DE LAVAL
RIV. SAULT-AU-COCHON
N
RIV. PORTNEUF
PORTNEUF
ILE DU HAVRE
0 1 2
SCALE IN MILES
WATER SAMPLING STATIONS
MOBILE LABORATORY
QUEBEC HEALTH DEPT.
CLAM SPECIMENS

FIGURE 5
RIV LAVAL TO PORTNEUF

1959 BACTERIOLOGICAL SURVEYS

Q-6. Ste. Anne de Portneuf to Riviere Sault au Mouton

Homes along the shore are well back from the water, and the only possible sources of direct pollution noted were several homes and a school in the vicinity of sampling stations 30 to 35 inclusive (Fig. 6). The pollution potential of the area was considered to be negligible.

B. PRINCE EDWARD ISLAND AREAS

The Prince Edward Island investigation was concerned with four shellfish producing areas which, for purposes of discussion, may be subdivided into nine sectors:

P-1. Montague River, Sector A.

This sector was covered by sampling stations 1 to 14 inclusive and 50 to 59 inclusive, as shown in Fig. 7. The sector receives gross, direct pollution from the town of Montague (pop. 1,130) municipal sewerage system; two sewers, which service a hospital, a dairy, ten business establishments and many of the homes, empty into the River near the town bridge.

P-2. Montague River, Sector B.

While there is a considerable dilution factor, it is probable that some pollution from Sector A will reach Sector B (sampling stations 15 to 49 inclusive, Fig. 7). While no sewer outfalls were noted, it is probable that cottages and homes along the lower Montague River south shore will be a factor in the Sector B pollution picture. The town of Georgetown (pop. 750) undoubtedly contributes some pollution to Georgetown Harbour; past studies, however, have consistently failed to show this to be a significant factor in the pollution picture of the Sector. The remainder of the watershed is farm and bush land.

P-3. Brudenell River

The watershed of the Brudenell River is heavily wooded, and has few potential sources of pollution (sampling stations 60 to 90 inclusive, Fig. 7). The village of Brudenell (pop. 50) is located a considerable distance upstream from the study area, and there are no homes on the immediate shoreline of the River. The pollution potential of the Sector may thus be considered as negligible.

P-4. Cardigan River

This sector is represented by sampling stations 91 to 130 inclusive (Fig. 7). The village of Cardigan (pop. 210), 7 cottages near sampling stations 103 to 106, and the village of Newport (pop. 12) near station 128 constitute the only potential pollution sources in the Sector. The watershed is heavily forested, and the pollution hazard was deemed negligible.

P-5. Rustico Harbour

This sector is covered by sampling stations 3 to 15 inclusive (Fig. 8). The Harbour receives sewage, from the villages of North Rustico and Rustico Harbour, and fish wastes from wharf facilities along the waterfront. Homes along Mill Brook are potential pollution sources. It was concluded that the pollution status of the Harbour was moderate to gross.

P-6. Rustico Bay

This sector (sampling stations 1 to 3 and 16 to 93 inclusive, Fig. 8) appears to be free from significant sources of direct human pollution. Small communities on the upper reaches of the Hunter, Wheatley and Winter Rivers contribute pollution, directly or indirectly, to the Rivers; because of effective dilution, however, these streams have little effect on the bacteriological quality of the sea water in Rustico Bay. The watershed is

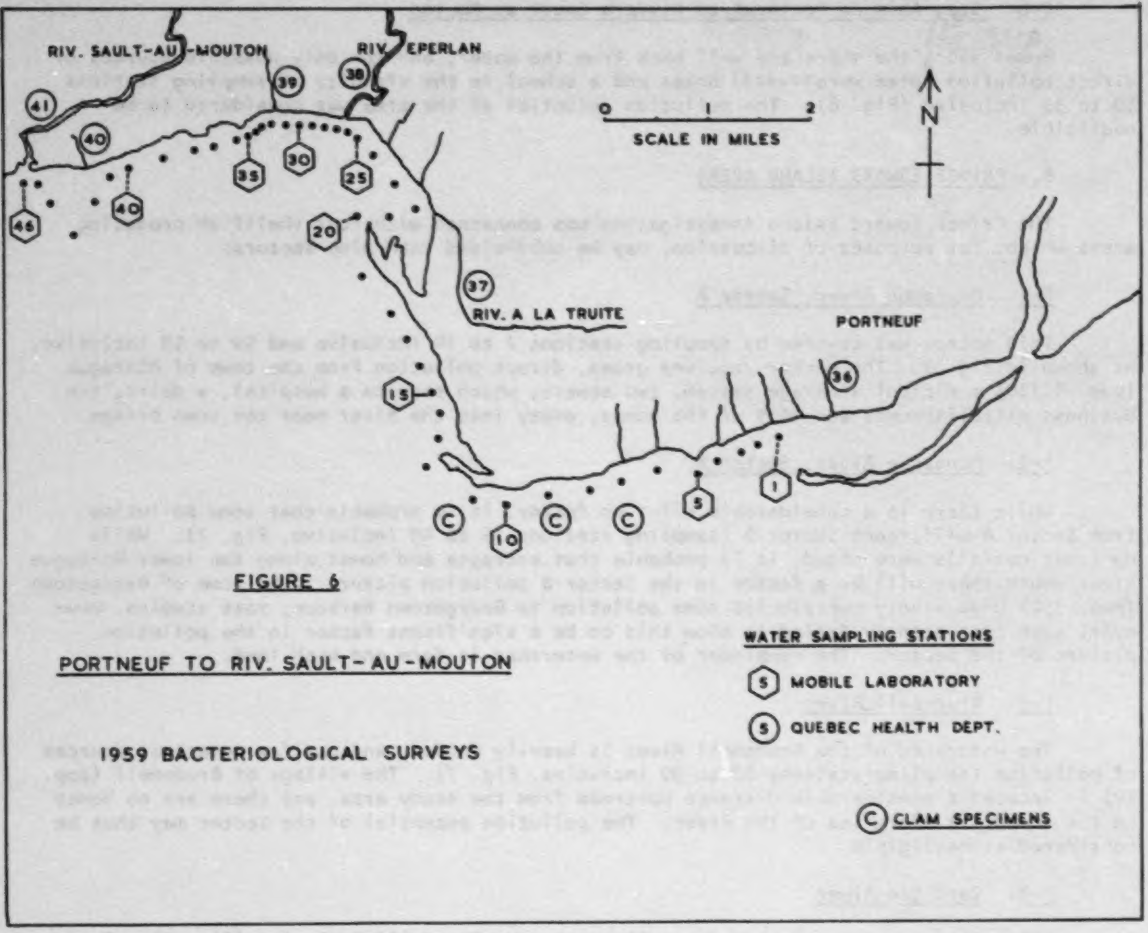

RIV. SAULT-AU-MOUTON
RIV. EPERLAN
41
39
38
40
0
1
2
SCALE IN MILES
N
35
30
25
40
45
20
37
RIV. A LA TRUITE
PORTNEUF
36
15
1
5
C
10
C
C
WATER SAMPLING STATIONS
5 MOBILE LABORATORY
5 QUEBEC HEALTH DEPT.
C CLAM SPECIMENS

FIGURE 6

PORTNEUF TO RIV. SAULT-AU-MOUTON

1959 BACTERIOLOGICAL SURVEYS

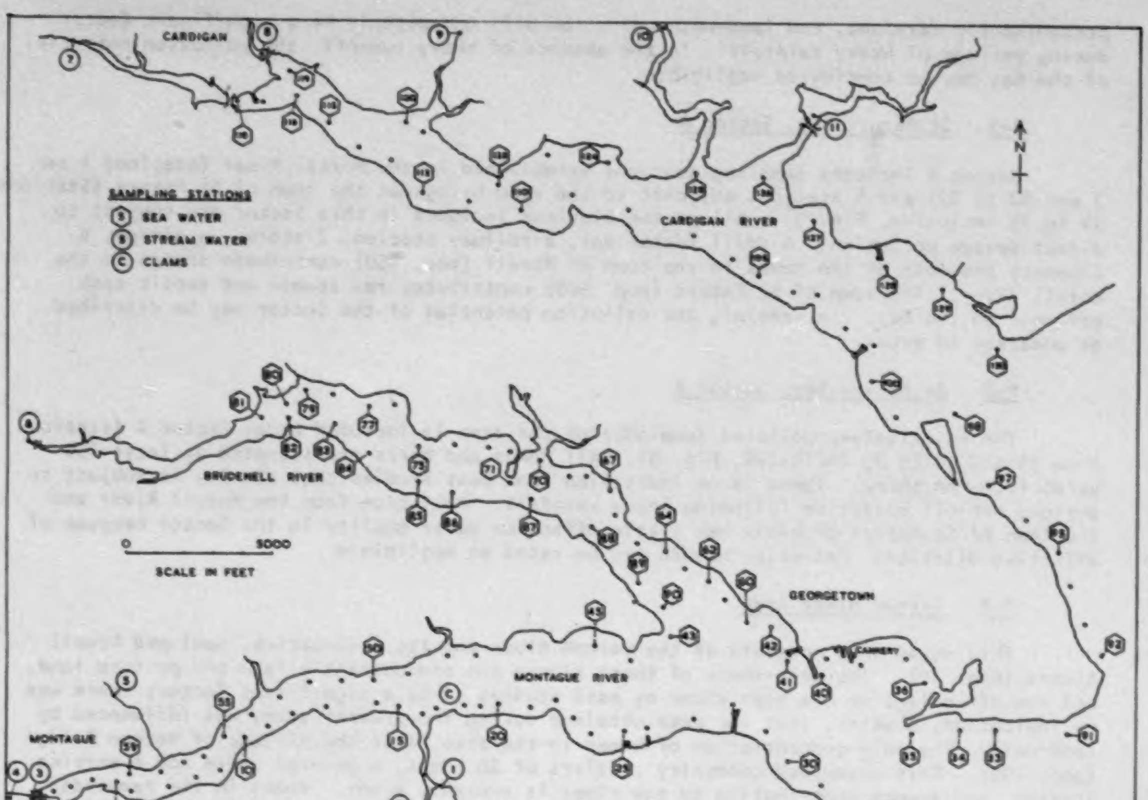

CARDIGAN
CARDIGAN RIVER
SAMPLING STATIONS
5 SEA WATER
5 STREAM WATER
C CLAMS
N
BRUDENELL RIVER
0
5000
SCALE IN FEET
GEORGETOWN
CANNERY
MONTAGUE RIVER
MONTAGUE

FIGURE 7

BACTERIOLOGICAL SURVEY, 1959. P.E.I. 12

predominantly farmland, and land-wash pollution will undoubtedly be a significant factor during periods of heavy rainfall. In the absence of heavy run-off, the pollution potential of the Bay may be considered negligible.

P-7. St.Peters Bay, Sector A

Sector A includes sampling stations established in the Morell River (stations 1 to 7 and 80 to 82) and 6 stations adjacent to the road bridge at the town of St.Peters (Stations 34 to 39 inclusive, Fig. 9). All of the Stations included in this Sector are subject to direct sewage pollution. A small restaurant, a railway station, 2 stores, a garage, a creamery and most of the homes in the town of Morell (pop. 350) contribute sewage to the Morell River. The town of St.Peters (pop. 500) contributes raw sewage and septic tank effluent to the Bay. In general, the pollution potential of the Sector may be described as moderate to gross.

P-8. St.Peters Bay, Sector B

The relatively-unpolluted remainder of the area is included under Sector B (stations 8 to 33 and 40 to 79 inclusive, Fig. 9). All homes and barns are situated at least 150 yards from the shore. There is no indication from past studies that the Bay is subject to serious run-off pollution following heavy rainfall. Pollution from the Morell River and the town of St.Peters probably has little effect on water quality in the Sector because of effective dilution. Pollution hazard may be rated as negligible.

P-9. Vernon River Area

This study area consists of the Vernon River and its tributaries, Seal and Orwell Rivers (Fig. 10). The watersheds of these rivers are predominantly farm and pasture land, and run-off pollution has been shown by past studies to be a significant factor; there was no indication, however, that the data obtained during the present study was influenced by land-wash. The only concentration of homes in the area is at the village of Vernon Bridge (pop. 100). This unsewered community consists of 20 homes, a general store and a service station, and sewage contribution to the river is probably minor. Homes in the remainder of the study area are well back from the water. Small streams entering the Orwell River are heavily polluted, probably from barnyard drainage, but had no demonstrable effect on the coliform content of Orwell River. Pollution levels in the area during the study could be described, in general, as negligible to slight.

Section III. Methodology

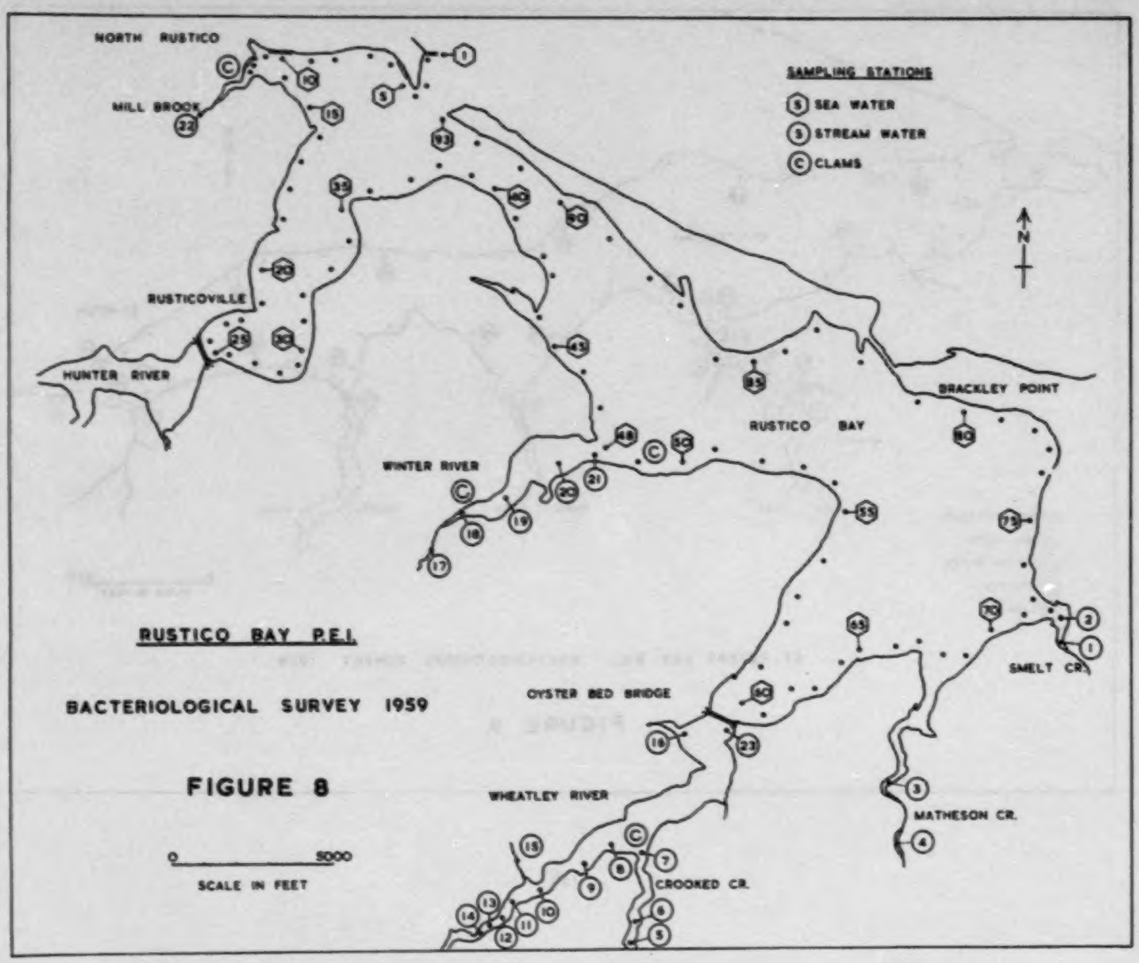
A. Laboratory Methods

1. Coliform Density Determinations

All specimens were tested by the methods outlined in the revised American Public Health Association "Recommended Procedures for the Bacteriological Examination of Shellfish and Shellfish Waters" for the determination of coliform densities. Coliform Confirmed Most Probable Numbers per 100 ml. or per 100 grams of sample were determined (Bacto-Lactose Broth, with five tubes in each of at least three dilutions, incubation at 35.5°C. for 24 and 48 hours, and confirmation of positive lactose broth cultures in Bacto-Brilliant Green Bile Broth).

2. E.C. Test Determinations

The presence of E.C. gas positive coliform strains was determined for all samples by the confirmation of all positive presumptive lactose broth cultures in Bacto-E.C. Medium, with incubation for 24 hours in a water-bath controlled at 44.5° ± 0.3° C. Results were expressed as Most Probable Numbers of E.C. gas positive coliform strains per 100 ml. or 100 grams of sample.

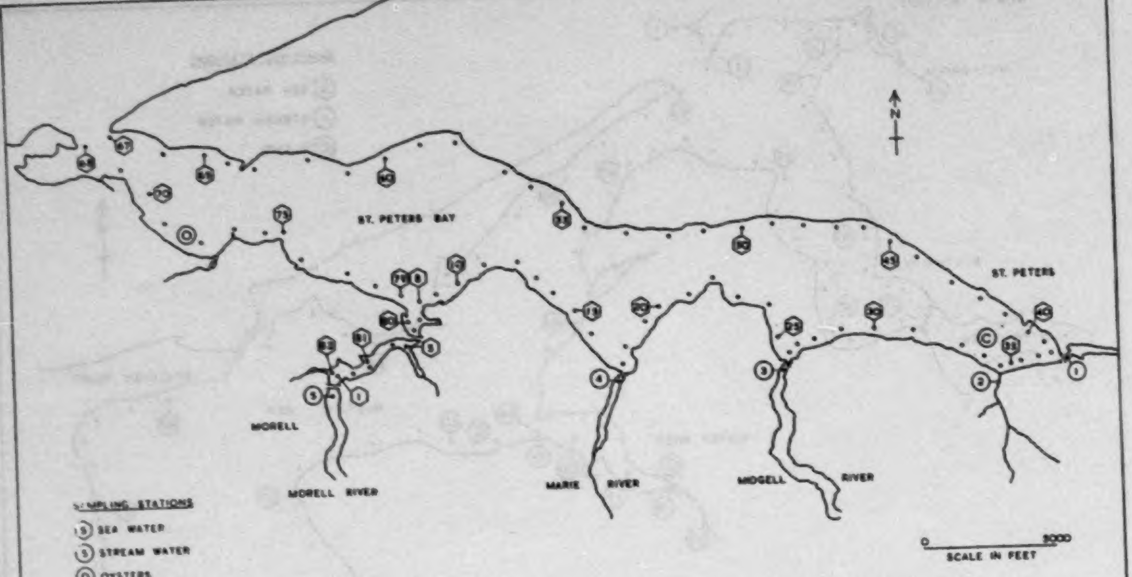


NORTH RUSTICO
MILL BROOK
RUSTICOVILLE
HUNTER RIVER
WINTER RIVER
RUSTICO BAY
BRACKLEY POINT
SMELT CR.
OYSTER BED BRIDGE
WHEATLEY RIVER
MATHESON CR.
CROOKED CR.
SAMPLING STATIONS
S SEA WATER
S STREAM WATER
C CLAMS
N
0
5000
SCALE IN FEET

RUSTICO BAY P.E.I.

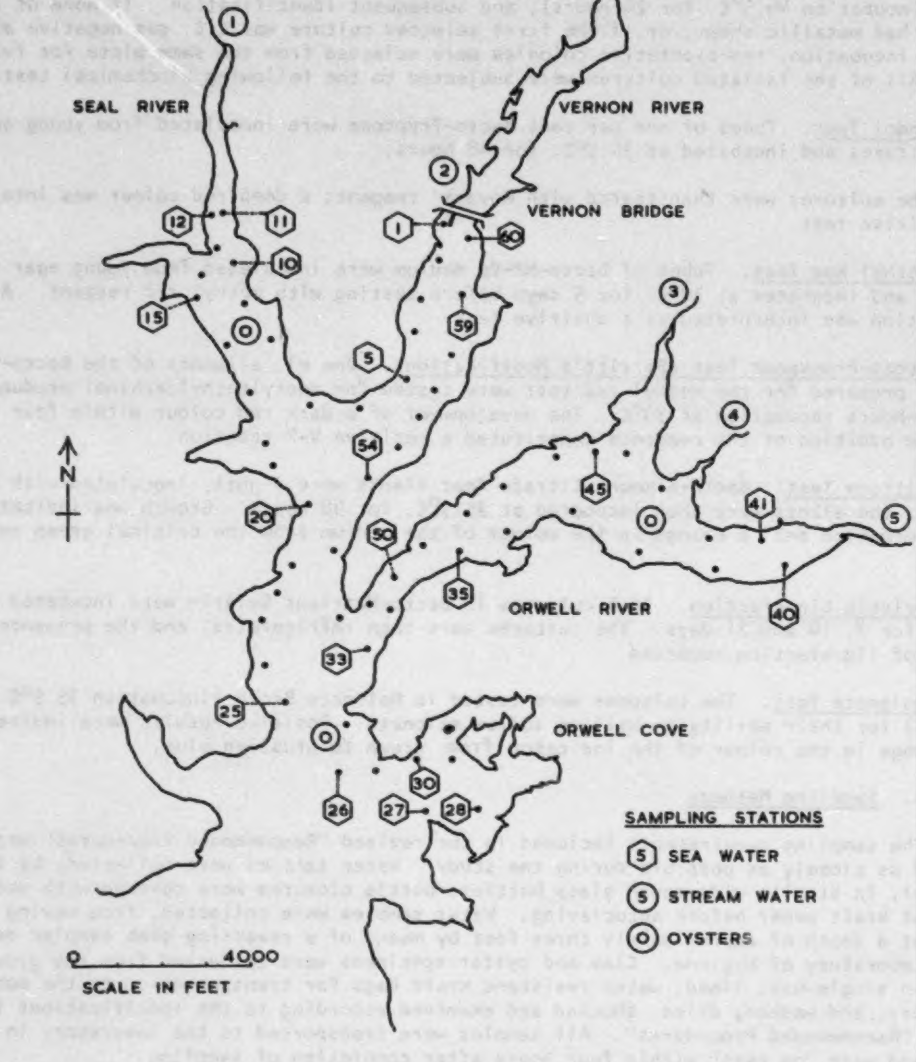
BACTERIOLOGICAL SURVEY 1959

FIGURE 8


N
ST. PETERS BAY
ST. PETERS
MORELL
MORELL RIVER
MARIE RIVER
MIDGELL RIVER
SAMPLING STATIONS
SEA WATER
STREAM WATER
OYSTERS
CLAMS
0
2000
SCALE IN FEET

ST. PETERS BAY P.E.I. BACTERIOLOGICAL SURVEY 1959

FIGURE 9


SEAL RIVER
VERNON RIVER
VERNON BRIDGE
ORWELL RIVER
ORWELL COVE
N
1
2
3
4
5
12
11
10
1
60
15
O
59
5
54
45
20
O
41
5
50
35
40
33
25
O
30
26
27
28
SAMPLING STATIONS
5 SEA WATER
5 STREAM WATER
O OYSTERS
0 4000
SCALE IN FEET

VERNON RIVER, P.E.I., BACTERIOLOGICAL SURVEY, 1959

FIGURE 10

3. Isolation and Identification of E.C. Gas Positive Strains

All E.C. gas positive cultures from all, or representative, samples from each study area were streaked on Bacto-E.M.B. Agar, and the plates were incubated at 35.5°C. for 24 hours. A discrete colony with metallic sheen was selected from each plate for transfer to Bacto-E.C. Medium (incubation 44.5°C. for 24 hours), and subsequent identification. If none of the colonies had metallic sheen, or if the first selected culture was E.C. gas negative after 24-hours incubation, representative colonies were selected from the same plate for further study. All of the isolated cultures were subjected to the following biochemical tests:

Indol Test. Tubes of one per cent Bacto-Tryptone were inoculated from young agar slant cultures and incubated at 35.5°C. for 48 hours.

The cultures were then tested with Kovacs' reagent; a deep red colour was interpreted as a positive test.

Methyl Red Test. Tubes of Bacto-MR-Vp Medium were inoculated from young agar slant cultures and incubated at 30°C. for 5 days before testing with methyl red reagent. A bright red reaction was interpreted as a positive test.

Voges-Proskauer Test (Barritt's Modification). One ml. aliquots of the Bacto-MR-VP cultures prepared for the methyl red test were tested for acetylmethylcarbinol production after 48-hours incubation at 30°C. The development of a dark red colour within four hours after the addition of the reagents constituted a positive V-P reaction.

Citrate Test. Bacto-Simmons Citrate Agar slants were lightly inoculated with the test cultures; the slants were then incubated at 35.5°C. for 48 hours. Growth was indicated by colony formation and a change in the colour of the medium from the original green to a deep blue.

Gelatin Liquefaction. Stab cultures in Bacto-Nutrient Gelatin were incubated at 35.5°C. for 7, 14 and 21 days. The cultures were then refrigerated, and the presence or absence of liquefaction recorded.

Malonate Test. The cultures were tested in Malonate Broth (incubation 35.5°C. for 48 hours) for their ability to utilize sodium malonate. Positive results were indicated by a change in the colour of the indicator from green to prussian blue.

B. Sampling Methods

The sampling requirements included in the revised "Recommended Procedures" were followed as closely as possible during the study. Water samples were collected, by laboratory personnel, in sterile wide-mouth glass bottles; bottle closures were covered with water-resistent kraft paper before autoclaving. Water samples were collected, from moving power boats, at a depth of approximately three feet by means of a reversing grab sampler designed at the Laboratory of Hygiene. Clam and oyster specimens were collected from the growing beds, placed in single-use, lined, water-resistant kraft bags for transportation to the mobile laboratory, and washed, dried, shucked and examined according to the specifications in the revised "Recommended Procedures". All samples were transported to the laboratory in cooler boxes, and were "on test" within four hours after completion of sampling.

Section IV. Bacteriological Data

A summary of the bacteriological data obtained for sea water and shellfish specimens from the fifteen study areas is reported in Table I.

The mean incidence of *E. coli* and other 44.5°C. E. C. gas positive coliform strains in sea water, as estimated by the E.C. Confirmation Test and expressed as a percentage of the total coliform density, was generally lower in Quebec than in Prince Edward Island study areas. The mean coliform: E.C. MPN ratios, as derived from the arithmetic average of the

TABLE I. SUMMARY, PERCENTILE LEVELS, COLIFORM AND E.C. GAS POSITIVE MOST PROBABLE NUMBERS, SEA WATER AND SHELLFISH

Area	No. of Samples	Most Probable Numbers per 100 ml.					
		COLIFORM			E.C.		
		50%	90%	10%	50%	90%	10%
Q-1	174	290	>1600	2	110	>1600	<1.8
Q-2	270	7.8	350	<1.8	2	70	<1.8
Q-3	246	79	>1600	11	23	540	4
Q-4	282	11	220	<1.8	2	22	<1.8
Q-5	252	22	1600	2	7.8	350	<1.8
Q-6	127	4.5	70	<1.8	2	13	<1.8
Clams	59	790	35000	68	330	8100	<18
P-1	144	350	>1600	49	130	1600	26
P-2	210	13	79	<1.8	4.5	33	<1.8
P-3	175	<1.8	6.8	<1.8	<1.8	4.5	<1.8
P-4	240	<1.8	11	<1.8	<1.8	7.8	<1.8
P-5	78	105	1600	6.8	29	920	2
P-6	480	2	17	<1.8	1.8	11	<1.8
P-7	96	70	920	13	33	240	4.5
P-8	396	4	23	<1.8	2	17	<1.8
P-9	360	4.5	23	<1.8	2	13	<1.8
Clams	12	1700	6000	45	790	4800	45
Oysters	14	78	390	20	20	220	<18

TABLE II. MEAN COLIFORM: E.C. MPN RATIOS, SEA WATER, SHELLFISH

Area	Pollution Status	Mean Coliform: E.C. MPN Ratios	
		All Samples Taken in Area	Samples used in Isolation Study
Q-1	Gross	1 : 0.51	1 : 0.56
Q-2	Negligible-Slight	1 : 0.42	1 : 0.70
Q-3	Slight-Gross	1 : 0.35	1 : 0.37
Q-4	Slight-Moderate	1 : 0.29	1 : 0.41
Q-5	Moderate-Slight	1 : 0.37	1 : 0.47
Q-6	Negligible	1 : 0.45	1 : 0.65
Clams		1 : 0.45	1 : 0.49
P-1	Gross	1 : 0.44	1 : 0.43
P-2	Slight	1 : 0.49	1 : 0.66
P-3	Negligible	1 : 0.81	1 : 0.87
P-4	Negligible	1 : 0.60	1 : 0.77
P-5	Moderate-Gross	1 : 0.51	1 : 0.51
P-6	Negligible	1 : 0.75	1 : 0.89
P-7	Moderate-Gross	1 : 0.57	1 : 0.50
P-8	Negligible	1 : 0.77	1 : 0.83
P-9	Negligible-Slight	1 : 0.66	1 : 0.83
Clams		1 : 0.73	1 : 0.75
Oysters		1 : 0.50	1 : 0.50

TABLE III (a). INCIDENCE OF E. COLI TYPE I AND OTHER 44.5°C. E.C. GAS POSITIVE COLIFORM BIOTYPES IN E.C. GAS POSITIVE CONFIRMATION TEST CULTURES, SEA WATER

Coliform (IMViC) Biotype	Area Q-1		Area Q-2		Area Q-3		Area Q-4		Area Q-5	
	No.	%	No.	%	No.	%	No.	%	No.	%
E. coli (++__)	117	90.7	113	91.1	160	82.9	180	83.7	169	80.5
Irregular II (_+__)	2	1.6	3	2.4	6	3.1	3	1.4	5	2.4
No. Cultures Containing "Fecal" Biotypes	119	92.2	116	93.5	166	86.0	183	85.1	174	82.9
A. aerogenes I (__++)	3	2.3	3	2.4	15	7.8	24	11.2	24	11.6
A. aerogenes II (+_++)	2	1.6	3	2.4	12	6.2	5	2.3	6	2.9
Intermed. I (_+_+)	1	0.8	0		1	0.5	2	0.9	2	1.0
Intermed. II (++_+)	2	1.6	1	0.8	2	1.0	0		3	1.4
Irregular V (__+_)	1	0.8	0				0		0	
MR+ VP+ (_+++;+++_)	1	0.8					0		0	
E.C. Gas Negative Coliform Biotypes	3	2.3	2	1.6	4	2.1	1	0.5	1	0.5
No. Cultures Which Did Not Contain "Fecal" Coliform Biotypes	10	7.8	8	6.5	27	14.0	32	14.9	36	17.1

TABLE III (b). INCIDENCE OF E. COLI TYPE I AND OTHER 44.5°C. E.C. GAS POSITIVE COLIFORM BIOTYPES IN E.C. GAS POSITIVE CONFIRMATION TEST CULTURES, SEA WATER

Coliform (IMViC) Biotype	Area Q-6		Area P-1		Area P-2		Area P-3		Area P-4	
	No.	%	No.	%	No.	%	No.	%	No.	%
E. coli I (++__)	90	91.8	99	84.6	203	90.6	99	98.0	132	97.1
Irregular II (_+__)	0		2	1.7	5	2.2	1	1.0	1	0.7
No. Cultures Containing "Fecal" Biotypes	90	91.8	101	86.3	208	92.9	100	99.0	133	97.8
A. aerogenes I (__++)	3	3.1	7	6.0	8	3.6	2	2.0	1	0.7
A. aerogenes II (+_++)	0		8	6.8	3	1.3	0		3	2.2
Intermed. I (_+_+)	0		1	0.9	2	0.9	0		0	
Intermed. II (++_+)	0		2	1.7	1	0.4	0		0	
MR+ VP+ (_+++;+++_)	4	4.1	3	2.6	1	0.4	0		0	
E.C. Gas Negative Coliform Biotypes	1	1.0	1	0.9	2	0.9	0		0	
No. Cultures Which Did Not Contain "Fecal" Coliform Biotypes	8	8.2	16	13.7	16	7.1	1	1.0	3	2.2

TABLE III (c). INCIDENCE OF E. COLI TYPE I AND OTHER 44.5°C. E.C. GAS POSITIVE COLIFORM BIOTYPES IN E.C. GAS POSITIVE CONFIRMATION TEST CULTURES, SEA WATER

Coliform (IMViC) Biotype	Area P-5		Area P-6		Area P-7		Area P-8		Area P-9	
	No.	%	No.	%	No.	%	No.	%	No.	%
E. Coli (++--)	218	96.0	32	86.5	163	90.1	43	91.3	306	98.1
Irregular II (-+--)	5	2.2	0		2	1.1	0		3	1.0
No. Cultures Containing "Fecal" Biotypes	223	98.2	32	86.5	165	91.2	43	91.3	309	99.0
A. aerogenes I (--++)	2	0.9	1	2.7	7	3.9	2	4.3	2	0.6
A. aerogenes II (+-++)	2	0.9	0		2	1.1	2	4.3	1	0.3
Intermed. I (-+-+)	0		0		2	1.1	0		0	
MR+ VP+ (-+++)	0		0		1	0.6	0		0	
E.C. Gas Negative Coliform Biotypes	0		4	10.8	5	2.8	0		0	
No. Cultures Which Did Not Contain "Fecal" Coliform Biotypes	4	1.8	5	13.5	16	8.8	3	6.5	3	1.0

TABLE IV. INCIDENCE OF E. COLI AND OTHER 44.5°C. E.C. GAS POSITIVE COLIFORM BIOTYPES IN E.C. GAS POSITIVE CONFIRMATION TEST CULTURES, SUMMARY

Incidence of Coliform	ALL PRINCE EDWARD ISLAND STUDY AREAS				
Biotypes	Polluted Sea Water	Unpolluted Sea Water	Clams	Oysters	Totals
Total No. E.C. Gas Positive Cultures Examined	200	1181	84	34	1499
Cultures Containing E.C. + E. coli Type I	174 (87.0%)	1121 (94.9%)	81 (96.4%)	33 (97.1%)	1409 (94.0%)
Cultures Containing E.C. + Irregular Type II	2 (1.0%)	17 (1.4%)	1 (1.2%)	0	20 (1.3%)
Cultures Containing E.C. + A. aerogenes I	10 (5.0%)	22 (1.9%)	0	1 (2.9%)	33 (2.2%)
Cultures Containing E.C. + A. aerogenes II	10 (5.0%)	11 (0.9%)	0	1 (2.9%)	22 (1.5%)
Cultures Containing Other E.C. + Biotypes	6 (3.0%)	7 (0.6%)	0	0	13 (0.9%)
Cultures from which no E.C. + Coliform Biotype Could be Isolated	5 (2.5%)	7 (0.6%)	2 (2.4%)	0	14 (0.9%)

TABLE V. INCIDENCE OF E. COLI AND OTHER 44.5°C. GAS POSITIVE COLIFORM BIOTYPES IN E.C. GAS POSITIVE CONFIRMATION TEST CULTURES, SUMMARY

Incidence of Coliform Biotypes	ALL QUEBEC STUDY AREAS				All Study Areas (Totals)
	Polluted Sea Water	Unpolluted Sea Water	Clams	Totals	
Total No. E.C. Gas Positive Cultures Examined	747	222	297	1266	2765
Cultures Containing E.C. + E. coli Type I	626 (83.8%)	203 (91.4%)	212 (71.4%)	1041 (82.2%)	2450 (88.6%)
Cultures Containing E.C. + Irregular Type II	16 (2.1%)	3 (1.4%)	3 (1.0%)	22 (1.7%)	42 (1.5%)
Cultures Containing E.C. + A. aerogenes I	66 (8.8%)	6 (2.7%)	51 (17.2%)	123 (9.7%)	156 (5.6%)
Cultures Containing E.C. + A. aerogenes II	25 (3.3%)	3 (1.4%)	17 (5.7%)	45 (3.6%)	67 (2.4%)
Cultures Containing Other E.C. + Biotypes	15 (2.0%)	5 (2.3%)	9 (3.0%)	29 (2.3%)	42 (1.5%)
Cultures from which no E.C. + Coliform Biotype Could be Isolated	11 (1.5%)	9 (4.1%)	3 (1.0%)	23 (1.8%)	37 (1.3%)

coliform: E.C. MPN ratios for the individual samples, also appear to indicate that E.C. gas positive strains constitute a smaller percentage of the total coliform density in "polluted" than in "unpolluted" areas (Table II). These data are particularly striking in P.E.I. areas, where there was a greater degree of uniformity of pollution levels within each area.

The incidence of E. coli type I and other coliform strains isolated from E.C. gas positive confirmation test cultures from sea water and shellfish specimens are recorded in Tables III (a, b and c), IV and V

E. coli type I was isolated from 82.2 and 94.0 per cent of the E.C. gas positive cultures examined in Quebec and P.E.I. respectively. In Quebec the per cent recovery of E. coli type I strains was lower for shellfish specimens than for sea water samples; the reverse was true in P.E.I. A.aerogenes type I (Irregular VI), A.aerogenes type II and Irregular type II were the most common other E.C. gas positive coliform types isolated.

Other E.C. gas positive coliform biotypes were found in association with E.coli type I in 28 E.C. gas positive cultures; similarly, E.C. gas negative coliform strains were isolated in association with E.C. gas positive strains from 61 E.C. cultures. The fact that mixed cultures do occur in E.C. gas positive tests precludes the direct use of any single screening test to further confirm the presence or absence of "fecal" types; it will be necessary to plate the cultures and to select colonies for further testing. It has also been shown that the production of a characterisitc metallic sheen on E.M.B. Agar is not a reliable criterion of the presence of "fecal" coliform types; many "non-fecal" coliform colonies produced typical sheen, while some E.coli type I and Irregular type II strains failed to do so. Sheen has also been shown to be a transient phenomenon with some E.coli type I strains.

It is difficult to explain our failure to isolate 44.5°C. gas positive strains from 37 (1. 3 per cent) of the E.C. gas positive cultures. It is possible that minority strains responsible for gas production in a mixed flora may have been overlooked at the time of isolation from the E.M.B. Agar plates. Most of the gas negative strains isolated from E.C. gas positive cultures had ++-- or --++ IMViC reactions; these may have been strains of E. coli type I or Irregular type VI which produced small amounts of gas in the E.C. confirmation cultures and failed to do so in pure culture. In any event, we contend that there can be no true "false positive" reactions under the terms of the E.C. Confirmation Test.

The E.C. Confirmation Test had a lower fidelity when applied to presumptive lactose broth cultures which were not recorded as gas positive until after 48 hours incubation. Such cultures, however, should be included in the test since "fecal" types still predominated in E.C. gas positive cultures derived in this manner.

No facilities were available for hydrographic study in any of the areas. Since the investigation included only a single summer season, there was no opportunity for the study of seasonal variation. There was no evidence that rainfall-induced runoff had any marked effect on the bacteriological data obtained during the investigation.

APPENDIX K

SANITARY SURVEY OF TILLAMOOK BAY AND SANITARY SIGNIFICANCE OF THE "FECAL" COLIFORM ORGANISMS IN SHELLFISH GROWING AREA WATERS

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Introduction

For years the shellfish control authorities in Oregon have considered the coliform index of pollution to be of questionable validity. In many instances, coliform MPN's were difficult to define due to the lack of correlation with known sources of pollution. Troubles were encountered interpreting dilution conditions in shellfish growing area waters and in fresh water streams contributing to growing areas. Interpretations were also affected by extreme high and low MPN's in areas not directly associated with sources of pollution. Therefore, evaluation and correlation of sanitary and bacteriological data almost always proved to be inconclusive.

For approximately two years, the staffs of the Shellfish Program and the Public Health Laboratory of the Oregon State Board of Health have collaborated with the staff of the Public Health Service Shellfish Sanitation Laboratory in a study to determine the sanitary significance of "fecal" coliform. In conjunction with the bacteriological study, comprehensive sanitary reconnaissance activities are being conducted in the contributing watershed areas.

Tillamook Bay was chosen as the major project area for the study. This choice was made primarily because Tillamook Bay is a shellfish growing area, is in relatively close proximity to the laboratory and has characteristics which tend to enhance such a study. Some of these characteristics are population concentrations, extensive livestock grazing areas and industrial sites. The area is also one of Oregon's largest coastal watersheds.

Description of Tillamook Bay and Watershed

A map of Tillamook Bay and the contributory watershed can be seen in Figure 1. Tillamook Bay, one of Oregon's largest bays, measures approximately four miles across at the widest part, and is fourteen miles long. With the exception of the channels, it is a very shallow bay, as the tidal areas are almost completely exposed at extreme low tides. The shellfish growing areas may be noted in Figure 1, and comprise approximately fifty percent of the available and potential shellfish growing sites in the bay. The oysters cultivated on the "beds" noted in Figure 1 are the Pacific oysters, *Crassostrea gigas*.

There are five major streams which collect and contribute surface runoff to Tillamook Bay. From North to South, the Miami River flows into the bay through a twelve-mile-long narrow valley which is located in the mountains in the northeast.

The Kilchis River flows from a valley east of Tillamook Bay. Its source is in the mountains, approximately fifteen miles from the bay. Both of these streams flow through forested, sparsely populated areas, considered to be of little sanitary significance.

The Wilson River is a stream of major sanitary significance. It is situtated near the northern edge of the Tillamook Valley, flowing from the east out of the coast range. The Tillamook Valley is situated east of the City of Tillamook extending seven miles to the foothills of the coast range. The Wilson River and the Tillamook Valley will be discussed under the section on Sanitary Reconnaissance.

The Trask River flows across the northern edge of the largest industrial site in the Tillamook Watershed. This industrial site is another densely populated area which lies south and east of the City of Tillamook. The City of Tillamook, also located on the Trask River, is the largest city in Tillamook County, having a population of 4,244. The sewage effluent from its treatment plant flows to the Trask River at a point approximately one and one-half miles southeast of Tillamook Bay.

Situated along the Tillamook River, South of the City of Tillamook, are farms and dairyland with several thousand head of dairy cattle. The Tillamook River flows past the west edge of the major industrial site and empties into Tillamook Bay in the same proximity as the Trask River. This is the area (approximately three miles southeast of the southern most end of Tillamook Bay) in which the sanitary reconnaissance is incomplete. Therefore, the results of the reconnaissance, in this specific area are inconclusive.

There are several population centers located around Tillamook Bay. (Note Figure I.)

Garibaldi, a city of 1,163, is located on the northern shore, approximately two miles east of the narrow mouth of Tillamook Bay.

The second area is an industrial site which lies just east of Garibaldi.

Hobsonville, a small community near the northern end of the east shore of Tillamook Bay, is situated approximately seventy-five feet above the level of the bay.

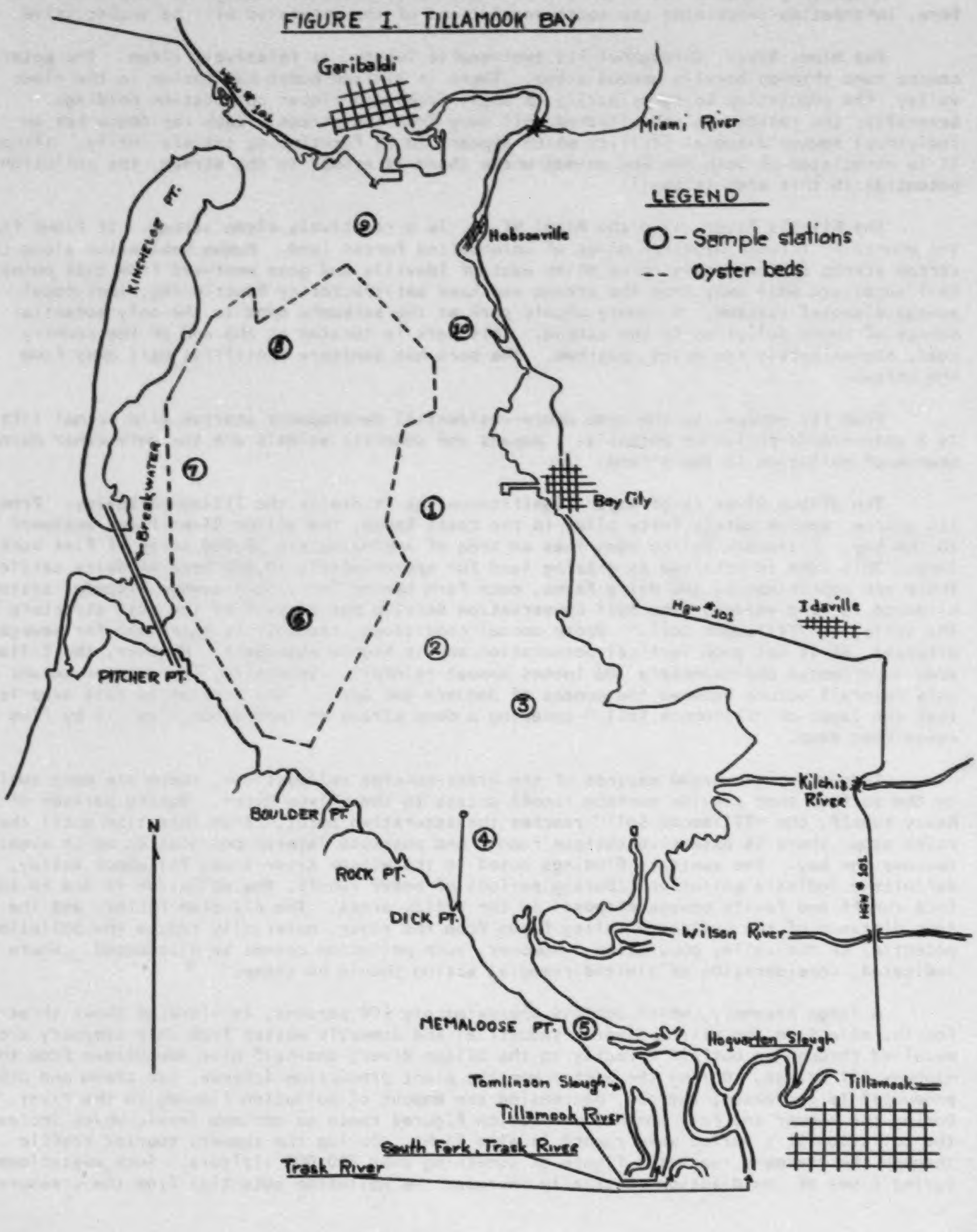
Bay City, with a population of 996, is situated about midway between the Northern and Southern ends on the east shore of the bay. There are two small streams, subject to surface runoff, ultimately reaching the bay.

Idaville is a community situated in an area which lies southeast of Bay City. It is located in a flat grassland used as pastureland for dairy cattle. This area borders a very low lying bottom land which is frequently flooded during heavy runoff periods when the Kilchis River overflows its banks. Although the pollution potential is present, the sanitary aspects regarding human pollution are slight; as, the main residential area is not situated in the bottom land.

Discussion concerning the City of Tillamook will follow in the section on Sanitary Reconnaissance. Tillamook is situated in the tide flats at the southeast end of Tillamook Bay. It is bordered on the north by Hoquarten Slough, on the East by the expansive low lying Tillamook Valley; on the south by the Trask River; on the west by several streams -- the Trask River, Tillamook River, and Tomlinson Slough; and on the northwest (approximately two miles) by Tillamook Bay.

As the mountains come directly to the shore of Tillamook Bay, the southwest shore from Memaloose Point to Pitcher Point is sparsely populated and heavily forested. (Please refer to Figure I.) The area from the Breakwater to Kincheloe Point is uninhabited. However, during the summer months, it is frequented by many sports clammers. The primarily human potential pollution factor in this area generally occurs during the tourist season (June through September).

FIGURE I. TILLAMOOK BAY


Garibaldi
Hwy # 101
Miami River
LEGEND
O - Sample stations
- - Oyster beds
Hobsonville
KINCHELOE PT.
BREAKWATER
9
10
8
7
1
Bay City
Hwy # 101
Idaville
6
2
PITCHER PT.
3
Kilchis River
BOULDER PT.
N
4
ROCK PT.
DICK PT.
Wilson River
Hwy # 101
MEMALOOSE PT.
5
Hoquarten Slough
Tomlinson Slough
Tillamook
Tillamook River
South Fork, Trask River
Trask River

Sanitary Reconnaissance

A sanitary reconnaissance of the watershed was conducted, though no completed; therefore, information concerning the southern-most end of the watershed will be inconclusive.

The Miami River, throughout its twelve-mile length, is relatively clean. The water course runs through heavily wooded areas. There is limited human habitation in the river valley, the population being primarily on small farms and timber corporation holdings. Generally, the residences are situated well away from the stream. Each residence has an individual sewage disposal facility which appears to be functioning satisfactorily. Although it is associated to both man and animal where there is access to the stream, the pollution potential in this area is small.

The Kilchis River, like the Miami River, is a relatively clean stream. It flows from the mountains through nineteen miles of uninhabited forest land. Human habitation along the stream starts approximately three miles east of Idaville and goes westward from that point. Residences are well away from the stream and have satisfactorily functioning, individual sewage disposal systems. A county picnic park at the stream's edge is the only potential source of human pollution to the stream. This park is located at the end of the country road, approximately ten miles upstream. The park has sanitary facilities well away from the stream.

From its source, to the area where residential development starts, wild animal life is a year-around-pollution potential. Humans and domestic animals are the only other major source of pollution to the stream.

The Wilson River is of major significance, as it drains the Tillamook Valley. From its source, approximately forty miles in the Coast Range, the Wilson River flows westward to the bay. Tillamook Valley comprises an area of approximately 30,000 acres of flat bottom land. This land is utilized as grazing land for approximately 10,000 head of dairy cattle. There are approximately 300 dairy farms, each farm having individual sewage disposal systems, situated in the valley. The Soil Conservation Service has classified the soil structure of the valley as "Tillamook Soil." Under normal conditions, the soil is excellent for sewage disposal, as it has good vertical percolation and is highly absorbent. However, the Tillamook area experiences approximately 100 inches annual rainfall. Generally, the greater amount of this rainfall occurs between the months of January and April. The problem in this area is that the layer of "Tillamook Soil," covering a deep strata of impervious clay, is by five to seven feet deep.

Throughout the broad expanse of the grass-covered valley floor, there are many swales in the surface that provide surface runoff access to the Wilson River. During periods of heavy runoff, the "Tillamook Soil" reaches the saturation point. From this time until the rains stop, there is extensive surface runoff and possible lateral percolation which eventually reaches the bay. The sanitary findings noted in the Wilson River area, Tillamook Valley, definitely indicate pollution. During periods of heavy runoff, the pollution is due to surface runoff and faulty sewage disposal in the valley areas. The dilution factor, and the long distance of the scattered valley farms from the river, materially reduce the pollution potential of the valley population. However, such pollution cannot be discounted. Where indicated, consideration of limited remedial action should be taken.

A large creamery, which employs approximately 800 persons, is situated about three-fourths mile from the Wilson River. Industrial and domestic wastes from this creamery are expelled through an outfall directly to the Wilson River, one-half mile downstream from the Highway 101 Bridge. During the winter months, plant production (cheese, ice cream and other products) is decreased; thereby, decreasing the amount of pollution flowing to the river. During the summer and fall months, production figures reach an optimum level, which increases the pollution at a period when runoff is also light. During the summer, tourist traffic through the creamery reaches a figure of something over 100,000 visitors. Such wasteflows during times of low dilution materially increase the pollution potential from the creamery.

The sanitary findings, relative to the creamery, decidedly indicate severe pollution at all times during the year. On this point, it is clearly indicated that immediate activity should be instigated to prevent the further discharge of sewage and wastes to the Wilson River.

The City of Tillamook is so situated that the pollution potential is critical. The city, at the south end of the bay, is so located that the surrounding area is tide land and salt marsh. The water table at any time of the year is rarely lower than ten feet. Because of this, high tides have a definite influence on sewage disposal systems. Approximately eighty-five percent of the city is sewered. A secondary sewage treatment plant has provisions for pre-chlorination and post-chlorination of effluent. At present, post-chlorination practices are being followed. During periods of heavy runoff, at times when there are six to eight foot tides, or both, severe infiltration of the sewer system occurs. This causes the plant to become hydraulically overloaded, forcing the undesirable use of the by-pass outfalls. During periods of light runoff, the plant experiences a loss of sewage solids, which results in subnormal operation of the digester component. This suggests that there is a problem of exfiltration in the sewage collection system. In some instances, even during periods of light runoff, the high tides cause infiltration of the sewage collection system. This results in the by-passing of sewage.

The reconnaissance indicated that all by-passing from the sewer system was into the Trask River. Hoquarten Slough does not receive sewage from the city system.

The sanitary findings in the City of Tillamook decidedly indicate pollution in the Trask River. Pollution from this source influences the sanitary condition of the bay. It is further evident that action needs to be taken to prevent, as far as possible, the problem of infiltration and exfiltration. The reconnaissance further indicates a definite need for the complete separation of the sanitary sewage system from the storm water system.

Although it is possible to say the Miami and Kilchis River areas are "relatively clean" and free of pollution, it must be recognized that the potential contributing population exists. It is further recognized that some pollution does exist and that, where indicated, some remedial action will be necessary.

The same problem exists at Garibaldi where there is a small sewage treatment plant. Approximately ninety percent of the population of Garibaldi is on a sewer system. Although the operation of the plant has been both effective and efficient, there still exists the possibility of a breakdown which will bring about the release of untreated sewage to the oyster beds. (See Station 8, 9, and 10 -- Figure I.)

In the other areas, such as Hobsonville, Bay City, and Idaville, the application of the words "relatively clean" can be given. These areas, from the standpoint of their potential contributing population, are considered as relatively good. Nevertheless where the need is indicated, corrective action must be taken.

Throughout this paper, relatively little has been said concerning the bay usage and the potential contributing population. Tillamook Bay is basically non-commercial. There is no shipping, except for the commercial fishing fleet based at Garibaldi. This fleet uses the main channel from Garibaldi to the Pacific Ocean. No commercial fishing is done in Tillamook Bay. The only exception to the aforementioned statement is a group of three of four commercial "crabbers" who run trap lines in the bay in the vicinity of Stations 7, 8, 9, and 10. The remainder of the bay is utilized by the oysterman. The only other usage of the bay is during the tourist season, and this is very limited. Determinations made on the bay indicate only a very slight pollution potential, certainly not enough to warrant any extensive surveillance.

Bacteriological Survey

Sample stations on Tillamook Bay are shown in Figure I. The number of samples necessary to give a valid representation of the area to be studied and the selection of sample stations were both considered. It was determined that ten sample stations would be selected. In so far as possible, samples would be collected from each station at four stages of tide. The stations were selected by conferring with Doctor Wayne V. Burt, Head of the Department of Oceanography, Oregon State University, concerning the critical placement of the ten sample stations. The stations were placed where they would best correlate with potential sources of pollution. Note in Figure I that the sample stations are so located that they transect each sector in the bay from the north to the south end. Since the tidal area is shallow, there is little, if any, stratification of water. There is a tumbling or rolling action of the water which tends to indicate a nearly complete mixing of fresh and salt water. Water samples are collected entirely at the bottom.

For this study, water samples were collected from February 1960 through May 1961. Bacteriological surveys were conducted in February, June, October, and December 1960. From January 1961 through October 1961, monthly bacteriological surveys have been conducted. Since the various seasons, climatic variations, and tidal conditions may be readily observed, more representative sampling will result from this type of coverage. Samples were submitted to the following bacteriological tests:

1. Multitube test for coliform organisms.
2. E. C. liquid confirmatory test for "fecal" coliform organisms. (Incubation in water bath at 44.5°C.)
3. Completed E. C. IMVIC test for classification of coliform types.

The American Public Health Association's "Recommended Procedures for the Bacteriological Examination of Sea Water and Shellfish," were used in the conduct of all tests.

Results

Table I presents a comparison of coliform and "fecal" coliform MPN's at various station groups in Tillamook Bay. All values were taken from logarithmic probability plots at various percentile levels. Each of the station groups noted in Table I show a definite relationship to potential sources of pollution, seasonal trends, and tidal influences.

Table I is set up to demonstrate station groups and seasonal relationships. Except for the MPN's noted at the ninety and ninety-five percentile levels at Stations 1, 2, and 6, the coliforms are lower during light runoff periods than during heavy runoff periods. This is explained, however, by the fact that these MPN's occurred during a low ebbing tide in the proximity of the Wilson River which is the receiving stream for industrial and domestic effluent from a large creamery. Within the restricted confines of the Wilson River during that period, MPN's from a sample station, situated approximately 200 yards below the creamery outfall, resulted in indeterminate values greater than 110,000. Dilution in the stream and the effect of low tide in the bay supports the occurrence of high MPN's. (See Figure II, Light Runoff.)

It must also be noted that while coliform counts are high, the MPN's of "fecal" coliform at Stations 1, 2, and 6, are materially lower. When compared to heavy runoff, the MPN's of "fecal" coliform at Stations 1, 2, and 6 are proportionately higher than coliform MPN's.

Stations 3, 4, and 5 are closest to the major sources of pollution. Here, again is the observation of higher MPN values during light runoff periods at the ninety-five percentile level. (see Figure III, Light Runoff.)

TABLE I. COLIFORM AND "FECAL" COLIFORM MPN'S AT VARIOUS STATION GROUPS IN TILLAMOOK BAY

		Probability Percentiles				
		Coliform				
Stations	Runoff Conditions	5	10	50	90	95
1,2,6	Light	3.1	7.2	130.0	2500.0	5200.0
	Heavy	60.0	90.0	360.0	1500.0	2200.0
3,4,5	Light	<3.0	2.5	78.0	2400.0	6200.0
	Heavy	88.0	140.0	740.0	3800.0	6000.0
7,8,9,10	Light	<3.0	2.0	23.0	220.0	520.0
	Heavy	28.0	42.0	170.0	660.0	1100.0
		"Fecal" Coliform				
		5	10	50	90	95
1,2,6	Light	2.2	3.9	31.0	240.0	430.0
	Heavy	11.0	18.0	110.0	660.0	1100.0
3,4,5	Light	<3.0	<3.0	15.0	350.0	900.0
	Heavy	21.0	38.0	270.0	1400.0	3300.0
7,8,9,10	Light	<3.0	<3.0	6.0	82.0	170.0
	Heavy	5.8	10.0	66.0	420.0	720.0

TABLE II. PERCENT CONFORMITY WITH RECOGNIZED STANDARDS AND THE RELATIONSHIP OF "FECAL" COLIFORM TO THE PRESENT STANDARDS

Percent Attainment - Coliform

Stations	Runoff Conditions	3.0	7.8	23	70	230	700
1,2,6	Light	---	11	22	38	60	75
	Heavy	---	---	---	7	34	73
3,4,5	Light	---	---	2	5.9	9.8	33 *
	Heavy	---	---	---	3.0	18	48
7,8,9,10	Light	14	28	50	71	89	96
	Heavy	---	---	3.5	22	62	91

"Fecal" Coliform

Stations	Runoff Conditions	3.0	7.8	23	70	230	700
1,2,6	Light	7.5	20	42	70	89	96
	Heavy	---	---	13	38	70	91
3,4,5	Light	2.0	6.5	18	38	65	84
	Heavy	---	---	6	18	46	74
7,8,9,10	Light	36	55	74	89	96	---
	Heavy	1.8	7.5	23	52	81	95

* PERCENTAGES TAKEN FROM ARRAYED MPN'S, NOT FROM PROBABILITY PLOTS

Stations 1, 2, and 6, and Stations 3, 4, and 5 are situated in the southern half of the bay; and, all are in proximity to the major sources of pollution. Stations 3, 4, and 5 routinely show higher MPN's of coliform and "fecal" coliform than do Stations 1, 2, and 6.

Stations 7, 8, 9, and 10 are in areas distant from the sources of pollution. As such, lower MPN's of coliform and "fecal" coliform are routinely observed. It must be noted, however, that even this area is influenced by the pollution from the southern end of Tillamook Bay.

Without exception, the MPN's of "fecal" coliform for all station groups and all climatological conditions are lower than the reported coliform MPN's.

Table II presents a comparison of coliform and "fecal" coliform at various station groups in Tillamook Bay using various MPN levels. The readings are expressed in percentages. The percentage levels were obtained from logarithmic probability plots and do not express a median or mean. They express the percent conformity of the total station group with the recognized MPN standards. This table indicates that during periods of light runoff, coliform MPN's are lower than during periods of heavy runoff. It should be pointed out that both Table I and Table II show a definite lack of conformity with recognized standards as they relate to the coliform index. Both tables indicate a rather significant degree of variability in coliform MPN determinations; whereas, the results with "fecal" coliform are less variable and coincide more closely with known sources of pollution. seasonal conditions and tidal changes.

Tidal conditions have a definite influence on the distribution of coliform and "fecal" coliform throughout Tillamook Bay. This may be more clearly observed by referring to Figures 2, 3, and 4. The bar graphs provide another means of evaluating the differences between the median MPN's of coliform and "fecal" coliform during light and heavy runoff periods at four different stages of tide. The stages of tide are indicated by letters -- LP (After Low), HM (Before High), HP (After High), LM (Before Low). It will be noted that each set of graphs uses a different range of MPN's. The median MPN's noted in each set of graphs are indicative of the respective station groups and are representative of their proximity to known sources of pollution. In all cases, with one exception, the periods just after and just before low tide have the highest MPN's of both coliform and "fecal" coliform. The lone exception occurred during the heavy runoff period on Stations 7, 8, 9, and 10 on an ebbing tide shortly after high tide. In this instance the "fecal" coliform also responded, reaching a magnitude greater than the fecal coliform at either stage of low tide. The reason for this exception is not clearly definable. Although Stations 7, 8, 9, and 10 are situated well away from the main sources of pollution and are considered to be the "cleanest" stations, pollution, however remote, may have reached and influenced them. It is also important to indicate that heavy runoff, or periods of high dilution, do not necessarily mean that shellfish growing in those areas will be free of fecal pollution.

Figures 2, 3, and 4 as with Tables I and II, show conclusively that the coliform MPN's in any given condition exceed the MPN's of "fecal" coliform. This fact further substantiates the theory that the "fecal" coliform more precisely define pollution, in a manner that shellfish control authorities can more effectively evaluate shellfish growing areas in the determination of approved areas.

Table III presents another aspect of the same problem by showing the coliform, "fecal" coliform relationships which are expressed in terms of the percent of the coliform MPN's that are "fecal." In keeping with Tables I and II, as well as Figures 2, 3, and 4, the same criteria for comparison of coliform and "fecal" coliform is used. This table brings out the fact that relatively high percentages of "fecal" coliform are found in Tillamook Bay waters. These results correlate well with the results noted in the other tables, in that heavy runoff periods and low tides render high MPN levels. It further correlates with the other data, that coliform MPN's show a higher degree of variability than "fecal" coliform MPN's. The data supports the theory that "fecal" coliform provides a more accurate measurement of the degree of pollution and provides a more efficient means of evaluating the suitability of shellfish growing area waters for approval.

TABLE III. COLIFORM, "FECAL" COLIFORM RELATIONSHIPS AT VARIOUS STATION GROUPS DURING DIFFERENT TIDAL STAGES

	Percent "Fecal" Coliforms			
	Heavy Runoff Period			
Stations	**LP**	**HM**	**HP**	**LM**
1,2,6	52	33	29	20
3,4,5	39	30	39	25
7,8,9,10	35	39	35	39
	Light Runoff Period			
	LP	**HM**	**HP**	**LM**
1,2,6	9.6	9.6	16	14
3,4,5	20	19	7.8	19
7,8,9,10	9.6	---	---	21

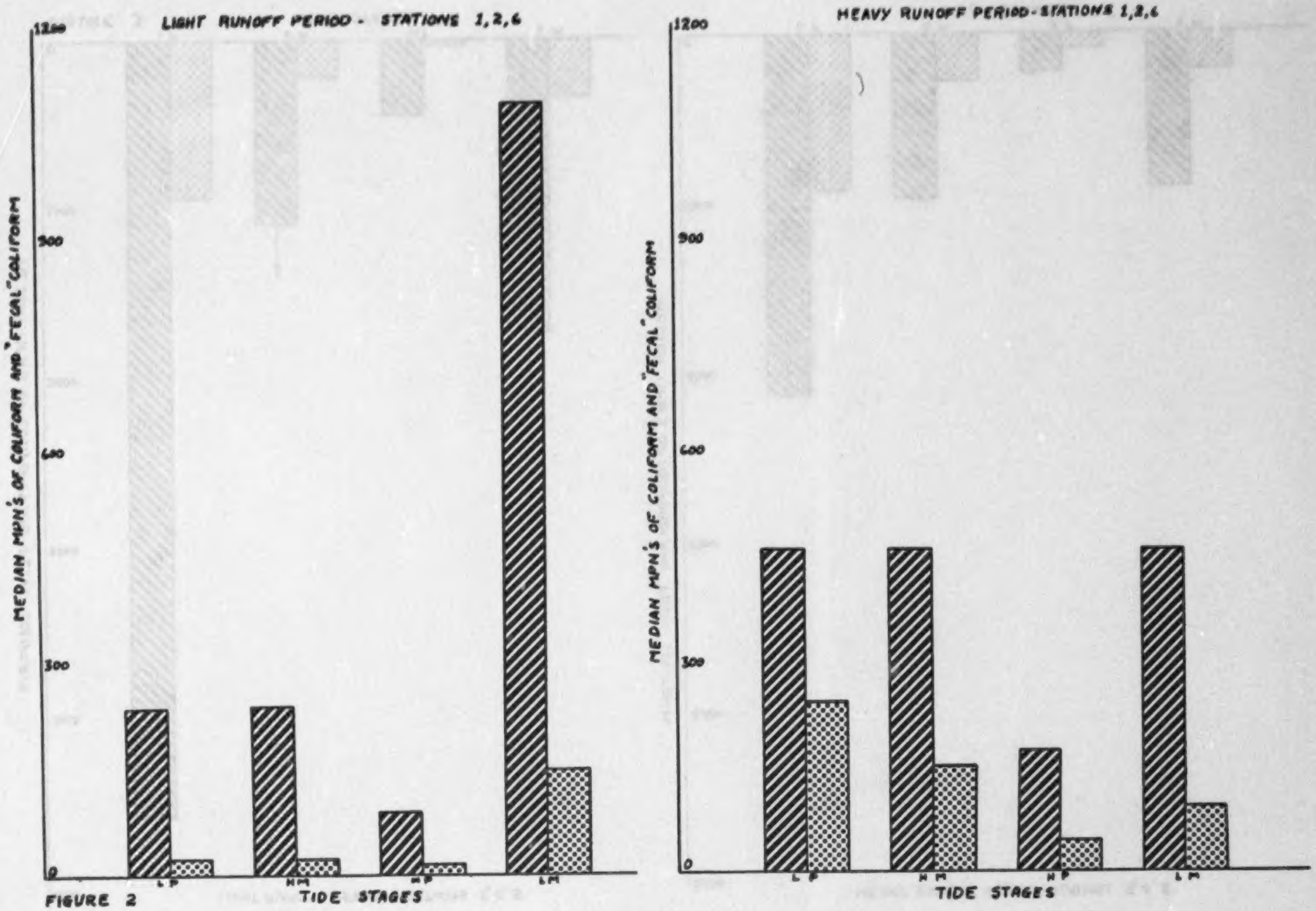

LIGHT RUNOFF PERIOD - STATIONS 1,2,6
MEDIAN MPN'S OF COLIFORM AND "FECAL" COLIFORM
1200
900
600
300
0
L P
H M
H P
L M
TIDE STAGES
HEAVY RUNOFF PERIOD-STATIONS 1,2,6
MEDIAN MPN'S OF COLIFORM AND "FECAL" COLIFORM
1200
900
600
300
0
L P
H M
H P
L M
TIDE STAGES

FIGURE 2

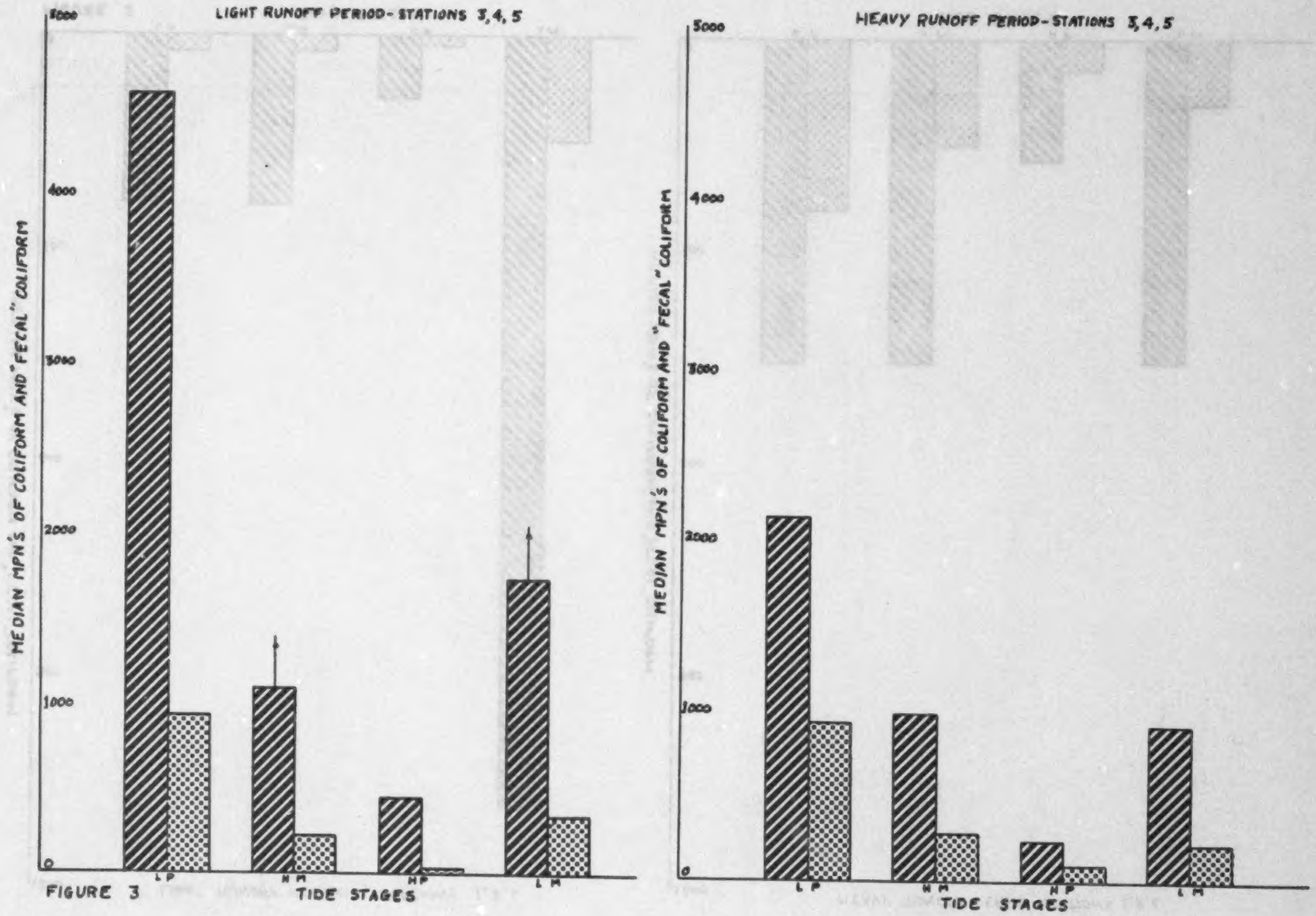

LIGHT RUNOFF PERIOD-STATIONS 3,4,5
MEDIAN MPN'S OF COLIFORM AND "FECAL" COLIFORM
5000
4000
3000
2000
1000
0
L P
H M
H P
L M
TIDE STAGES
HEAVY RUNOFF PERIOD-STATIONS 3,4,5
MEDIAN MPN'S OF COLIFORM AND "FECAL" COLIFORM
5000
4000
3000
2000
1000
0
L P
H M
H P
L M
TIDE STAGES

FIGURE 3

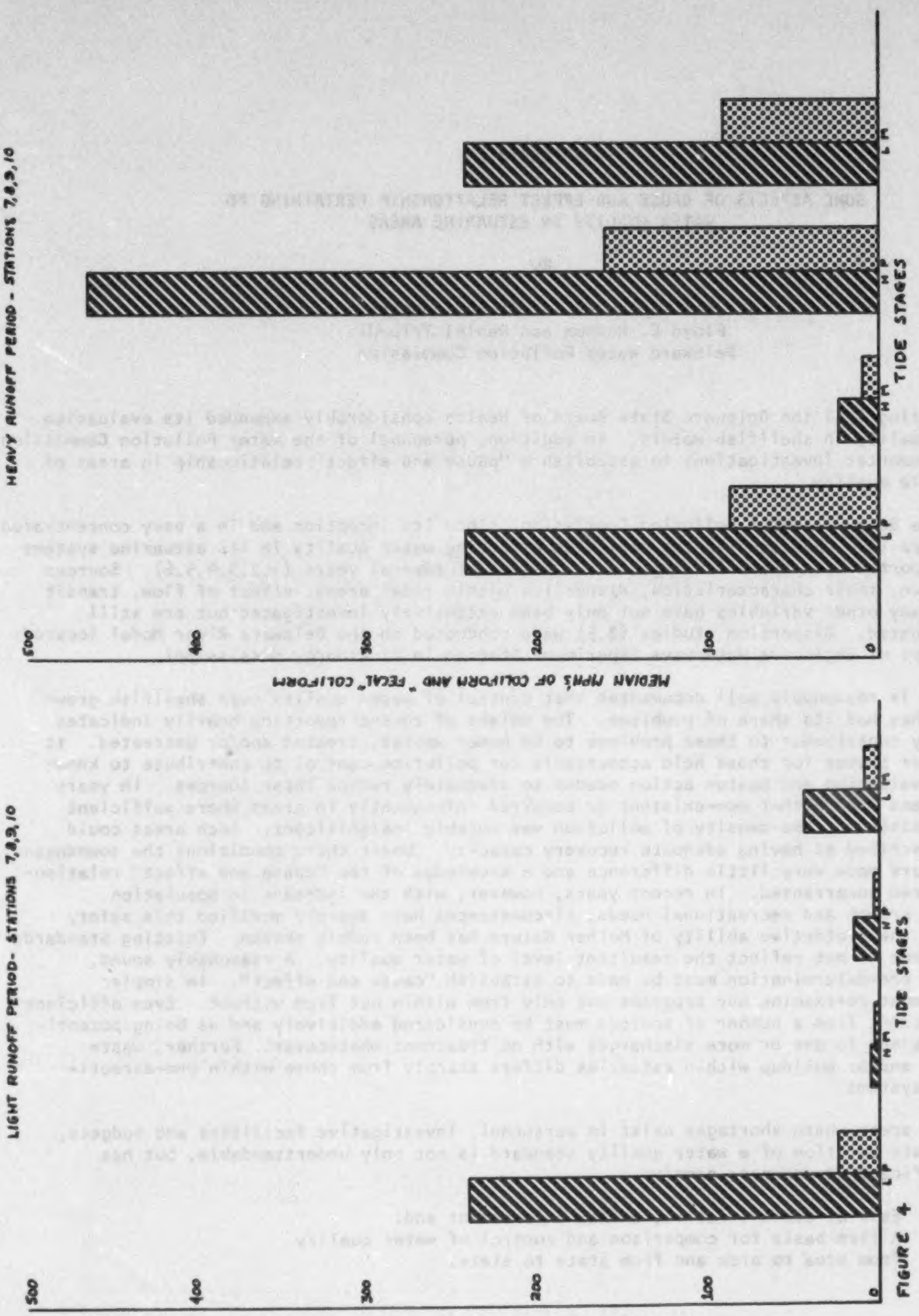

HEAVY RUNOFF PERIOD - STATIONS 7,8,9,10
MEDIAN MPN'S OF COLIFORM AND "FECAL" COLIFORM
500
400
300
200
100
0
L P
H W
H P
L W
TIDE STAGES
LIGHT RUNOFF PERIOD- STATIONS 7,8,9,10
MEDIAN MPN'S OF COLIFORM AND "FECAL" COLIFORM
500
400
300
200
100
0
L P
H W
H P
L W
TIDE STAGES

FIGURE 4

APPENDIX L

SOME ASPECTS OF CAUSE AND EFFECT RELATIONSHIP PERTAINING TO WATER QUALITY IN ESTUARINE AREAS

By

A. Joel Kaplovsky, J. Steven Anderson
Floyd E. Hudson and Daniel Tripodi
Delaware Water Pollution Commission

During 1961 the Delaware State Board of Health considerably expanded its evaluation of water quality in shellfish waters. In addition, personnel of the Water Pollution Commission made supplemental investigations to establish a "cause and effect" relationship in areas of questionable quality.

The Delaware Water Pollution Commission, since its inception and in a very concentrated effort since 1956, has studied the variables affecting water quality in its estuarine systems and has reported upon these findings over a period of several years (1,2,3,4,5,6). Sources of pollution, their characteristics, dispersion within tidal areas, effect of flow, transit time and many other variables have not only been extensively investigated but are still being evaluated. Dispersion studies (2,5) were conducted on the Delaware River Model located at the Corps of Engineers Waterways Experiment Station in Vicksburg, Mississippi.

It is reasonably well documented that control of water quality over shellfish growing areas has had its share of problems. The weight of recent reporting heavily indicates the primary contributor to these problems to be human wastes, treated and/or untreated. It would appear proper for those held accountable for pollution control to contribute to knowledge of evaluation and hasten action needed to adequately reduce these sources. In years past problems were either non-existent or occurred infrequently in areas where sufficient dilution existed or the density of pollution was notably insignificant. Such areas could also be described as having adequate recovery capacity. Under these conditions the soundness of a Standard made very little difference and a knowledge of the "cause and effect" relationship appeared unwarranted. In recent years, however, with the increase in population, industrial growth and recreational needs, circumstances have sharply modified this safety factor and the protective ability of Mother Nature has been rudely shaken. Existing Standards for treatment do not reflect the resultant level of water quality. A reasonably sound, scientific pre-determination must be made to establish "cause and effect". In simpler terms, we must re-examine our programs not only from within but from without. Even efficient waste treatment from a number of sources must be considered additively and as being potentially equivalent to one or more discharges with no treatment whatsoever. Further, waste dispersion and/or buildup within estuaries differs sharply from those within one-directional-flow systems.

In areas where shortages exist in personnel, investigative facilities and budgets, the immediate adoption of a water quality standard is not only understandable, but has many significant advantages, namely:

1. Ease of administration, and/or enforcement and;
2. A firm basis for comparison and control of water quality from area to area and from state to state.

The adoption of a uniform standard, nationally, would have similar advantages.

In many cases, standards of water quality were developed from "the best available information". These requirements may have been based upon laboratory controlled experiments or an accumulation of analyses made over an extended period of time and, unfortunately, under widely variable conditions.

Decisions with regard to shellfish growing areas, a few hundred yards below a large sewer outfall, or a growing area remote from any human or animal sources, exhibiting little or no contamination, requires very little imagination or intelligence to evaluate. Unfortunately, present day problems do not arise from these extreme conditions, but from conditions which may be affected by an intermediate level of pollution. In the latter instance the adoption of a proper standard requires not only judgment, but a knowledge of the "cause and effect" relationships in order to make an adequate determination.

It is readily evident from the literature, that not only has much progress been made with regard to identification of fecal organisims and their isolation but that some knotty problems still exist in this regard. It appears the greatest efforts and hopes are directed toward finding the "cure-all" which could resolve quickly and simply the problems related to quality control evaluation. Pure research is always needed for further understanding and for achieving new and improved techniques. However, in our experience, we have found a notable "gap" when we apply the knowledge acquired in the universities and research laboratories to field conditions. Without a thorough knowledge of what the sample truly represents under field conditions, we would be nullifying the best of the laboratory achievements. We as government administrators must supplement the efforts of our academicians in the area of applied research far more then we have in the past.

The lack of sufficient effort in the direction of applied research is not too difficult to understand. Most states have inadequate budgets and too few trained personnel to do much more than adopt a standardized type program which can be maintained administratively more effectively within the budget and personnel available. However, the malignant outgrowth of this standardized approach usually results in a situation whereby, the adopted water quality requirements, based upon "best available information", become far more rigid than the Ten Commandments. Invariably efforts and interest to evaluate, improve and/or modify existing standards, based upon sound investigations, become stymied.

In reviewing the literature we have observed two important deficiencies with regard to research performed within estuarine waters, namely:

1. Compared to fresh water systems there is comparatively little published water quality study work on estuaries.
2. A great portion of the water quality information on tidal areas was accumulated without deference to direction of flow, wind, weather, temperature, runoff, and other variable influences.

It appears that only in recent years has there been any strong trends to record and/or consider the many variables inherent to movement within an estuary, and their possible influence upon the findings from an analytical measurement of water quality.

A normal estuary is subject to at least 28 variables. To this, one might add the location of a pollution source, unstandardized waste concentrations and composition, variable discharge rates and other man-made changes.

Unfortunately, very few estuarine systems have similar characteristics, and a universally rigid rule does not apply. We must understand our individual tidal system if we are to ever achieve a proper evaluation. There is an urgent need for considerable effort in the field of applied research.

THE PROBLEM:

When dealing with an estuary (1) one cannot emphasize too strongly that such a body of water has certain inherent complexities which are not present in streams unaffected by tidal action. Employing the old concept that "dilution is the solution to pollution" would lead to many erroneous conclusions. Due to the nature of water movement, in an estuary, certain conditions are created in regard to pollution buildup and recovery which need special considerations. Some of the more important observations are briefly summarized as follows:

A. An estuary receiving pollution in the form of a continuous discharge may result in a concentration buildup far exceeding that which can be anticipated from the uniform dispersion of the total daily loadings.

B. An estuary such as the Delaware River is, in essence, in a state of equilibrium and presently any daily loading is only a small portion of the existing accumulated concentration within the stream at any time.

C. Some of the more important factors of flow, transit time, temperature, and dispersion, have variable effects in different portions of the estuary. For example, the effect of increased flow upon water quality may not be the same in the upper reach of the estuary as compared to the lower reach.

D. The pattern of runoff and the total volume involved are extremely important with regard to the pollution buildup and recovery.

E. Sediment problems will always be present, either through direct solids discharge, erosion, or chemical coagulation and/or flocculation of industrial-type wastes. Regimentation of natural runoff pattern may reduce the effect of scour.

F. The total volume of water within a drainage basin area is extremely important, in that each gallon of water carries dissolved oxygen and has a definite recovery ability. Any decrease in volume will lessen the total recovery capability. Benefits derived from controlled flow and/or diversion must be carefully weighed against the disadvantages, in specific areas, with regard to pollution abatement.

G. Transit time, which represents the average movement time of a contaminant, is of comparatively long duration, pollution-wise, which, in turn, makes many estuarine systems large waste recovery systems.

H. Due to the long transit time inherent in many estuaries, controlled and/or augmented flow in the upper basin may result in a change of the pollution regimen and of salinity movement. Any short term estimate to determine the maximum concentration buildup of either pollution or salinity can result in extremely low values. A continuous and gradual buildup of both pollution and salinity will take place over an extended period of time until equilibrium is reached. This maximum may be affected not only by the rate of discharge but also by the total volume passing down the estuary over an extended period of time.

It has been assumed that certain of our closed shellfish beds, exhibiting coliform levels in excess of the acceptable standards, are contaminated by municipal waste discharges existing upstream. We were disturbed, however, by the fact that in two such closed areas in Delaware the municipal pollution sources were not large, by present standards, and were located 8.5 and 10.4 miles upstream from the beds as shown in Figure I (Estuary A) and Figure II (Estuary B) respectively. These curves represent typical patterns existing in several of our estuaries. A plot is shown of the E.C. MPN/100 ml. vs. distance in miles. Sampling was conducted in accordance with the modified same-slack technique (4) developed and reported upon by the Delaware Water Pollution Commission. In the two examples shown, samples were collected at low-water slack in lieu of high-water slack because the water quality over the shellfish beds under the former would not be influenced by the cleaner Bay waters adjacent thereto. Two significant points may be observed from each of these presentations, namely:

1. There is a continuous decline in E.C. MPN downstream from the municipal sources reaching minimum levels upstream from the shellfish bed areas, and thereafter, a rise in E.C. MPN count occurs over each bed and;

2. The time of transit in the Estuary A from the municipal source has been estimated to be 14 days, at a minimum, and 19.5 days for Estuary B, at a minimum.

It may be surmised that one of two conditions induce the high downstream MPN levels. There was either a secondary growth of E.Coli or another source of pollution in the lower reach was accountable for the rise in the MPN's. In view of the comparatively long transit time from the source of municipal waste it appeared unlikely that a secondary growth could be the culprit. Consequently, extensive and careful sanitary surveys of the areas downstream were made and revealed the absence of human or significant animal discharges in either Estuary A or B. However, the bacteriological downstream-patterns persist. If the level of fecal contamination must be determined and a sanitary survey made of a part of any evaluation, it follows that an acceptable coliform MPN level and a questionable sanitary survey could constitute at least a conditionally-approved area. However, if a sanitary survey shows conditions of unquestionable excellence and at the same time the coliform MPN exceeds the adopted standards, are we to assume that the sanitary survey has no significance and that the coliform MPN findings become the deciding factor? This creates somewhat of an ambiguity, in that, if no human or animal sources are accountable downstream the E.C. MPN observed have their origin from an unknown source other than what is normally associated with carriers of pathogenic organisms. It would be presumptious to assume that the E.C. MPN measured in these particular estuaries were erroneous or do not originate from fecal sources. Obviously, there must be a reason for the E.C. levels observed and the need for the investigations reported upon below.

INVESTIGATION RESULTS:

The initial phase of the fecal streptococci investigations subjected all shellfish bed samples and potential areas to the biochemical reaction series of Kenner et.al. (7). It was also considered appropriate that the tributary flow to the main stem of estuaries A and B, located in close proximity to the shellfish beds within these streams be subjected to the quantification of streptococci in feces procedures of Kenner, et.al. Colonies on the KF media were selected at random and subjected to the biochemical reactions. Our initial objective was to differentiate, if possible, the source of the fecal streptococci attributable to human, fowl, cow, sheep, or pig discharges.

Appendant, hereto, is a series of drawings depicting the areas investigated, and the associated sanitary survey findings. In addition, a series of tables is included with the results of the bacteriological examinations. Figure 1 presents the general location of the sanitary and bacteriological survey areas within the State of Delaware. Sketches A, B, and C, show detail of the sanitary survey work within Duck Creek, Slaughter Creek, Cedar Creek, Crooked Gut, Gregos Canal, Prime Hook, Old Mill and Canary Creeks. Sketches A-1, B-1, B-2, B-3, C-1, and C-2, depict the bacteriological survey and station locations in each of the above areas. Tables 1 through 3 present the findings in the Duck Creek area. Table 4, shows the data for Crooked Gut, and Table 5 for Gregos Canal. Tables 6, 7, 8, and 9, present bacteriological findings for Cedar Creek, Slaughter Creek, Prime Hook Creek, and Canary-Old Mill Creeks respectively.

The initial phase of our investigations emphasized the application of the procedure of Kenner et.al. (7) to actual field conditions. A specific determination of fecal streptococci and their application to the interpretation of pollution problems was the objective. Appropriate samples were filtered through the membrane filter and cultivated on KF streptococcus media. A representative number of colonies was picked

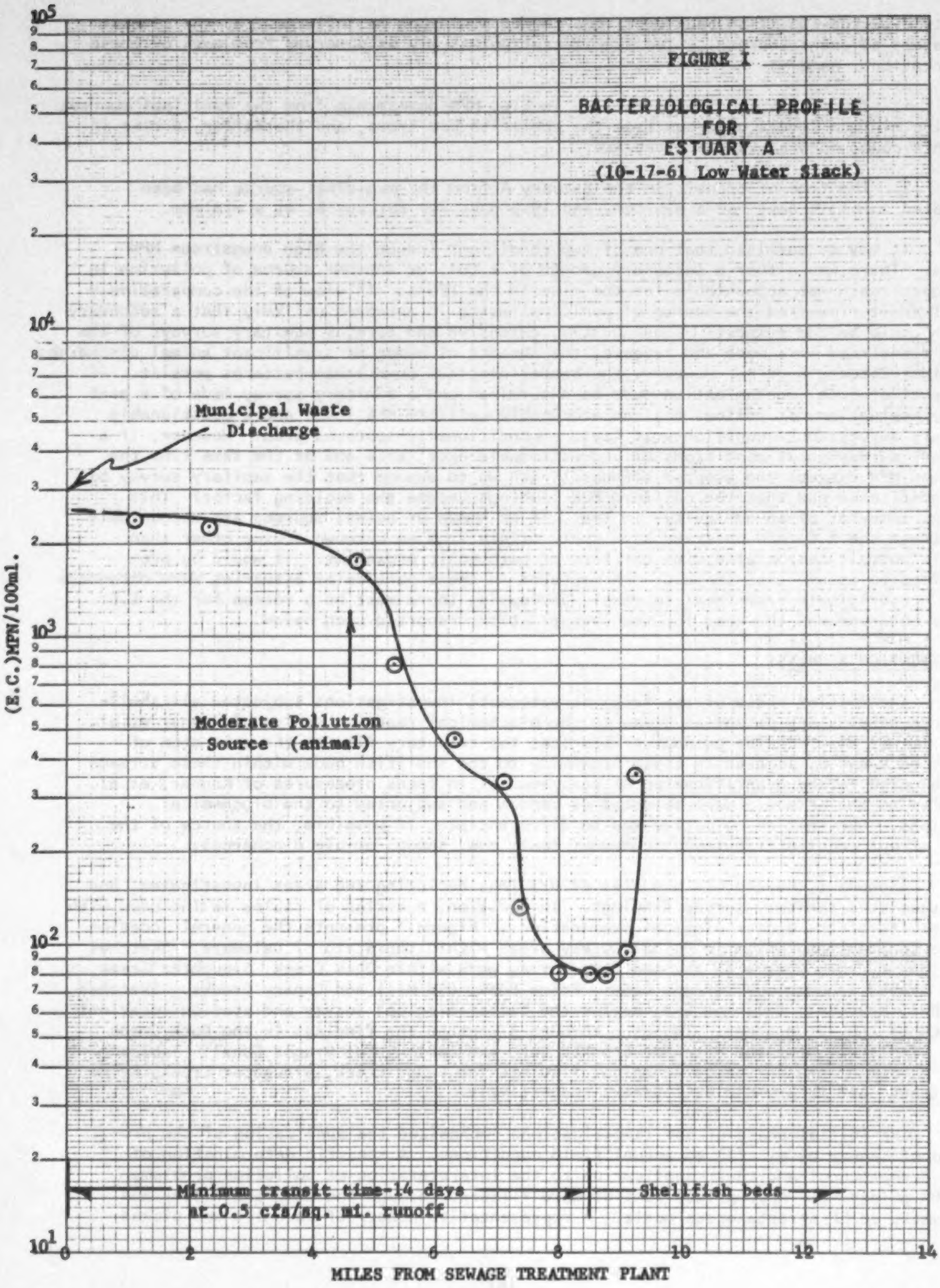


FIGURE I

BACTERIOLOGICAL PROFILE FOR ESTUARY A (10-17-61 Low Water Slack)

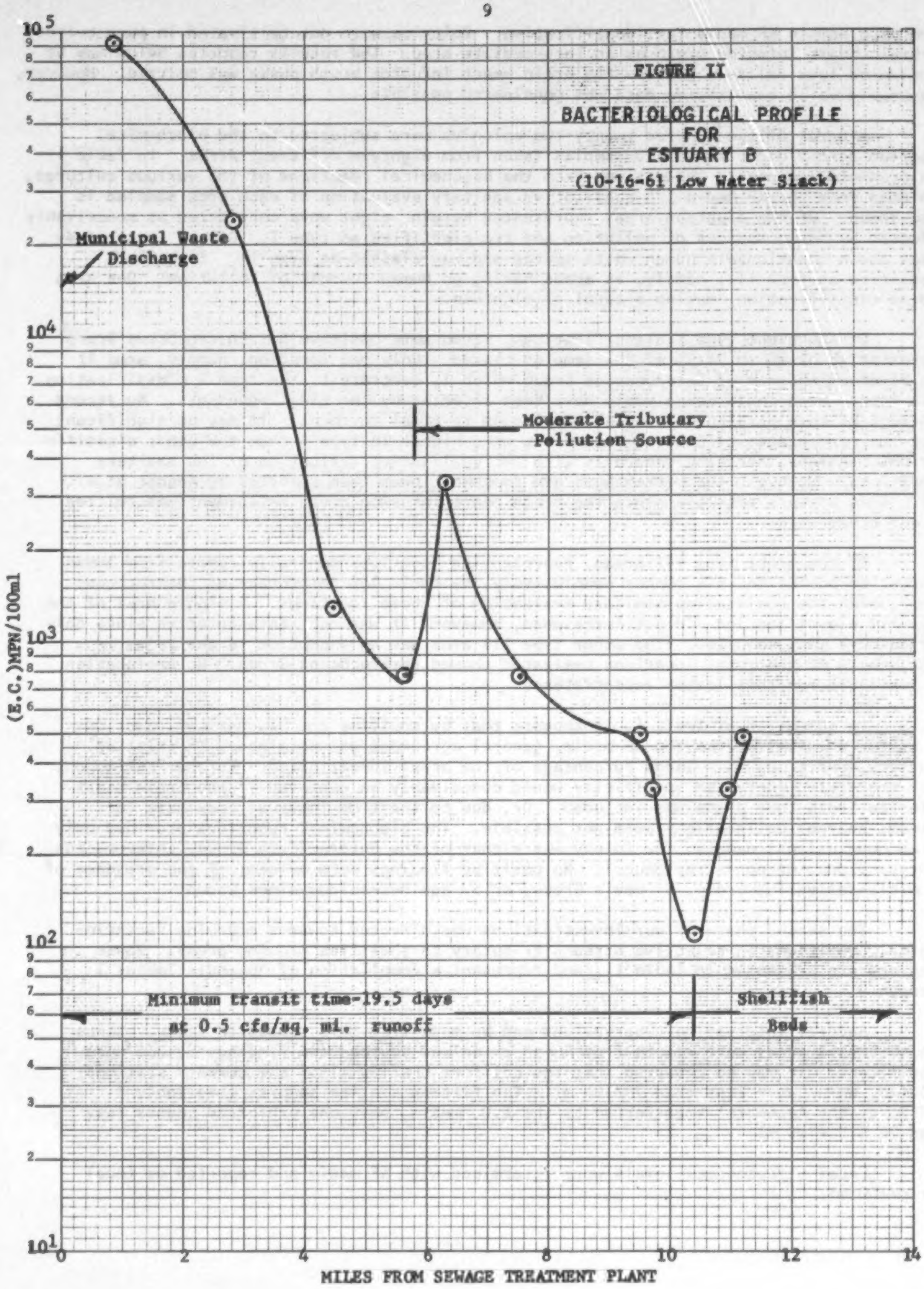


FIGURE II

BACTERIOLOGICAL PROFILE FOR ESTUARY B

(10-16-61 Low Water Slack)

from each sample KF media for identification. Colonies were not cultivated in pure culture in brain heart infusion broth as an intermediate step. The results reported below may be considered less reliable because the brain heart infusion broth phase was omitted. However, reasonably valid qualitative data was considered possible.

A total of two hundred twenty-two colonies were subjected to the biochemical reaction series and represented samples taken from eighteen different areas. In Table I are presented composite evaluations from the biochemical reactions of the various cultures, isolated from the KF media. A qualitative sanitary evaluation of each area sampled is also shown. Of the eighteen areas represented herein, eight were considered as conceivably affected by human sources of pollution and are classified as type I. One of these study areas has a questionable human waste source and was classed as type II. Eight were classified as type III; namely, as areas having no human sources of pollution. One area had no classification, making a total of eighteen.

Of the eight type I classifications, seven were positive for Enterococcus Group representing 42.8% to 71.5% of the samples tested. Only one location; namely, area 17 exhibited an absence of Enterococcus Group which is contrary to the type I classification. This latter area could conceivably have been affected by the tidal movements. No record was kept of this particular group of samples as to tidal movement. It may be significant that the percentages of Enterococcus Group were higher in type I than the other classifications, thereby, agreeing generally with the qualitative evaluation of the sanitary conditions. However, the percentages are somewhat lower than reported by Kenner et.al. (7). This appears possible since the brain heart infusion broth refinement was omitted in our study work.

Of the eight type III areas, representing locations generally remote from human sources of pollution, six concur conclusively, by their low percentage of Enterococcus Group, with the qualitative sanitary evaluation of these locations. A single area of the type III classification did not correspond, however, it was not representative since only one culture was evaluated. The other type III area was an exception to the rule, in that, 44.8% of the total locations evaluated showed Enterococcus Group. No explanation is available for this latter inconsistency.

In summation of Table I, it appears that by applying the biochemical reactions to cultures isolated from the KF media, general agreement was obtained with existing sanitary conditions in a large percentage of the areas investigated. Further refinement and, therefore, increased specificity would conceivably be achieved if the brain heart infusion broth tube phase was included. Or, due to the time interval from host to stream, sharper delineations were not possible. The biochemical reactions provided only three conclusions; namely, the colony was either of the Enterococcus Group, Enterococcus Biotype Group, or non-streptococci. No positive findings were evident of the presence of S. mitis-salivarius Group, S. bovis Group, or S. equinus-acidominimus Group.

The second phase of our investigations was directed towards studying bacteriological conditions in selective streams tributary to shellfish growing areas. These findings are presented in Table II, and represent a compilation of Appendix Tables 1 through 9.

Several interesting observations can be made from this composite Table. Since the selective areas were remote from human or animal sources the findings become more significant. It may be observed that the coliform MPN/100 ml of the seventy stations studied, were never less than 70/100 ml. The fifteen station samples (see Table 1 and 2, of the Appendix) transferred to the E.C. media, only one exhibited counts less than 70 E.C./100 ml.

Fecal streptococci levels were determined with KF media and reported as fecal strep/100 ml.

TABLE I

COMPOSITE EVALUATIONS FROM BIOCHEMICAL REACTIONS OF VARIOUS CULTURES ISOLATED FROM K. F. MEDIA

1961

Area No.	Type of Area Sampled	Enterococcus Group	Enterococcus Bio-type Group	Non Strep	Total
1.	I *	6	6	0	12
2.	III	0	3	4	7
3.	I	12	0	12	24
4.	III	0	0	3	3
5.	I	36	8	9	53
6.	I	5	2	0	7
7.	III	5	1	12	18
8.	III	1	1	2	4
9.	--	0	0	5	5
10.	III	1	0	0	1
11.	I	6	2	1	9
12.	I	6	6	2	14
13.& 14.	III & I **	4	4	6	14
15.	II	2	3	5	10
16.&17.	III & I ***	0	1	11	12
18.	III	13	2	14	29
TOTALS		97	39	86	222

* Probable Human Sources Type I
Questionable Human Sources Type II
No Human Sources Type III

** 7 Locations in Type III and 7 Locations in Type I

*** 4 Locations in Type III and 8 Locations in Type I

TABLE II

COMPOSITE OF BACTERIOLOGICAL SURVEY COVERING SELECTIVE AREAS TRIBUTARY TO SHELLFISH GROWING AREAS

1961

Appendix Table No.	No. of Stations	MPN/100 ml Range (1)	E.C./100 ml Range (2)	Strep/100 ml Range (3)
1	6	130 - 490	* 33 - 330	22 - 46
2	9	230 - 4900	230 - 490	16 - 162
3	9	230 - 4900	---	20 - 80
4	8	490 - 3100	---	20 - 315
5	5	221 - 4900	---	40 - 684
6	11	500 - 10,900	---	60 - 220
7	11	200 - 240,000	---	22 - 132
8	4	4900 - 13,000	---	47 - 88
9	(3	4600 - 24,000	---	574 - 712
	(4	700 - 3300	---	44 - 234
TOTAL	70			

* Only one value less than 79

(1) MPN/100 ml - BBG

(2) E.C./100 ml - E. C. Media

(3) Strep/100 ml - K. F. Media (Millipore Filter)

The fecal streptococci range varied from 16 to a maximum of 712 (see Table II). Since the fecal streptococci were measured employing the millipore filter, it is not appropriate to compare values with the MPN or the E.C. media, employing the multiple tube procedures.

In summation of Table II, the coliform MPN levels observed in these selective areas which are not only isolated but have excellent sanitary control, exhibited levels which could not meet the existing shellfish water standards. This same conclusion can be drawn from the E.C. MPN/100 ml. levels. Apparently, the bacteriological findings and the sanitary survey disagree sharply. In all the selected areas numerous waterfowl were frequently observed, and the droppings from these birds were evident throughout the area. There is little doubt that the high counts must be attributed, primarily, to the presence of waterfowl.

Since the coliform, E. Coli, and Fecal Streptococci levels are considered directly proportional to the possible presence of pathogenic organisms, and/or viruses, we must assume that these pathogens or viruses are potentially present in these isolated areas. The question arises, however, whether the relative levels of pathogens and viruses associated with feces include waterfowl. One might also raise the question whether or not undomesticated waterfowl are carriers of pathogenic organisms and viruses harmful to man. If no such relationship exists, migratory waterfowl areas having tributary flow to shellfish beds, may be the basis for closing a shellfish bed without due cause. It is conceivable that these natural habitat conditions existed for centuries. The establishment of a sound cause and effect relationship appears quite warranted.

SUMMARY OBSERVATIONS

1. Estuarine systems are invariably quite specific in their mixing characteristics dictating the necessity for a thorough understanding of known conditions such as tidal movement, runoff, waste discharge location, composition and buildup potential, transit time, temperature, and weather, for proper coordination and evaluation of study work in shellfish waters.

2. With the intention of being repetitious, "dilution is not the solution to pollution control".

3. Considerably more emphasis must be directed towards applied research by the Governmental agencies to more fully utilize and establish on a field basis, the findings of the pure researcher.

4. The quantification of fecal streptococci for application to the interpretation of pollution problems, employing KF media followed by the biochemical reaction series, when applied to field conditions in Delaware, resulted in reasonably good qualitative confirmation with known sanitary conditions. However, in areas with multiple sources of fecal streptococci, no group differentiation, other than Enterococcus and Enterococcus Biotype was evident.

5. It follows, with additional culture isolation or slight procedure modification, further differentiation may result and thereby become a useful tool of the applied researcher for the identification of pollution source.

6. Present standards of water quality for shellfish waters need further refinement and/or interpretation, particularly in areas affected by tributary drainage from waterfowl nesting grounds, or natural habitat, where coliform and E.C. MPN levels exceed the acceptable standards.

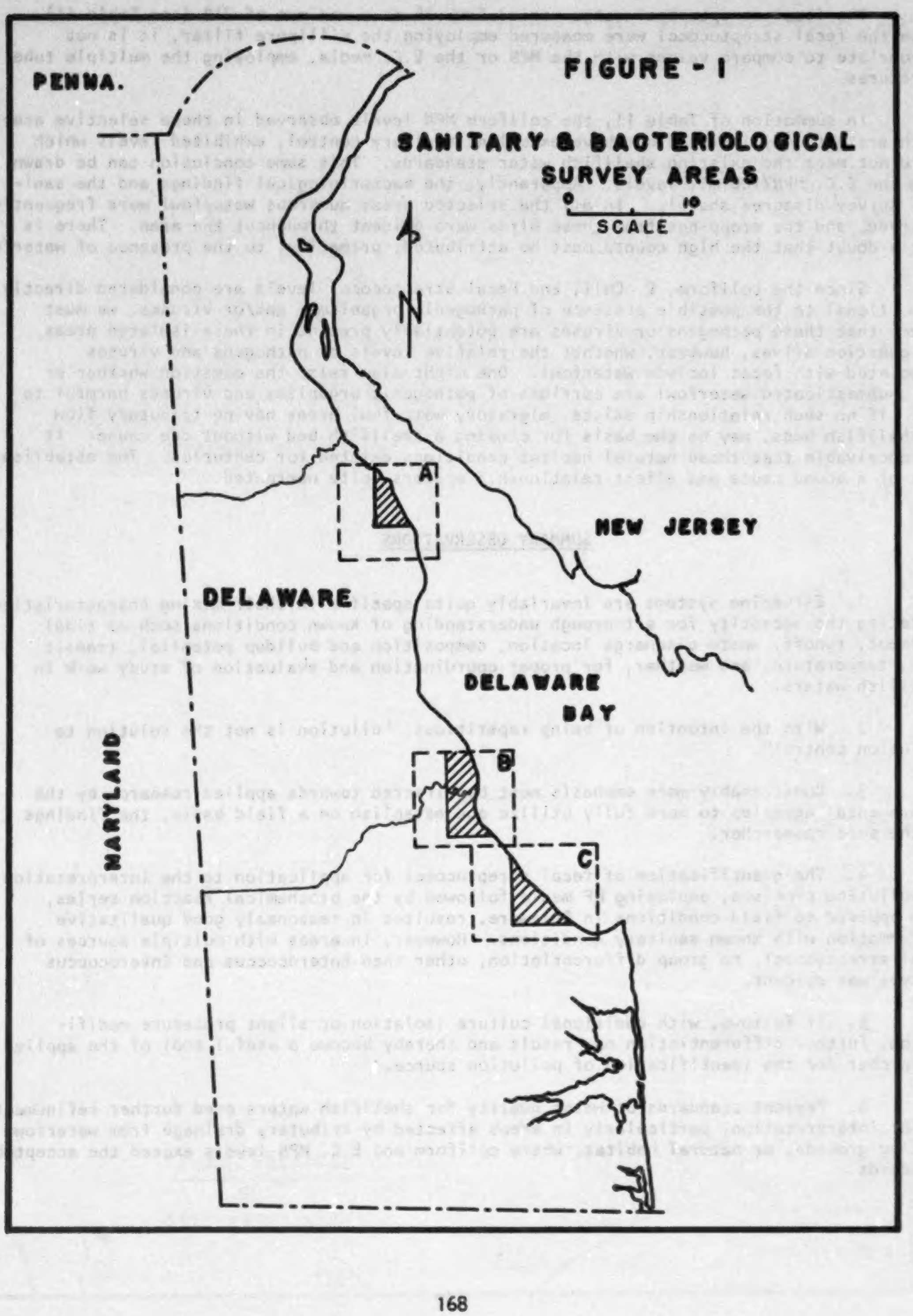
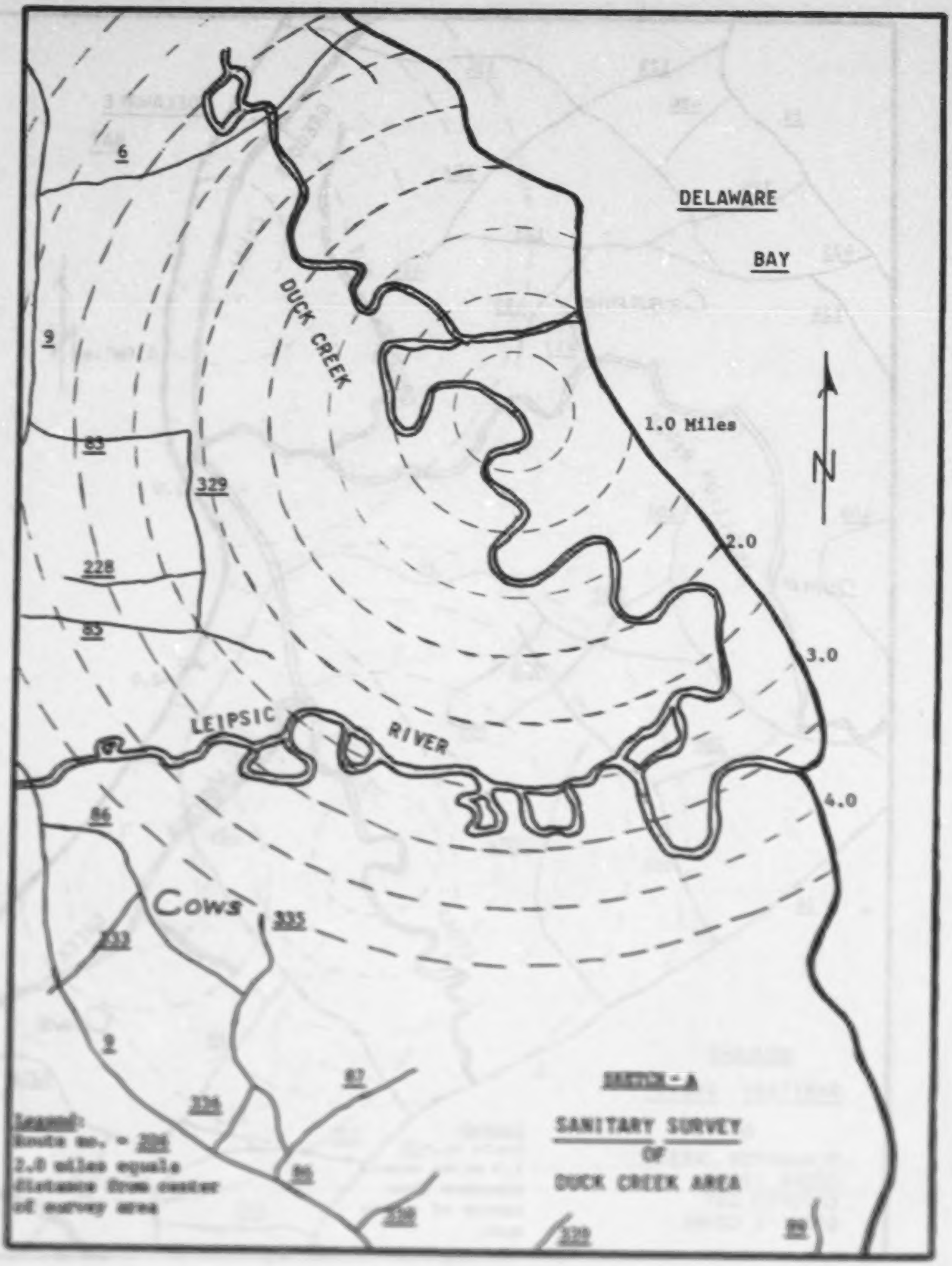


FIGURE - I

SANITARY & BACTERIOLOGICAL SURVEY AREAS

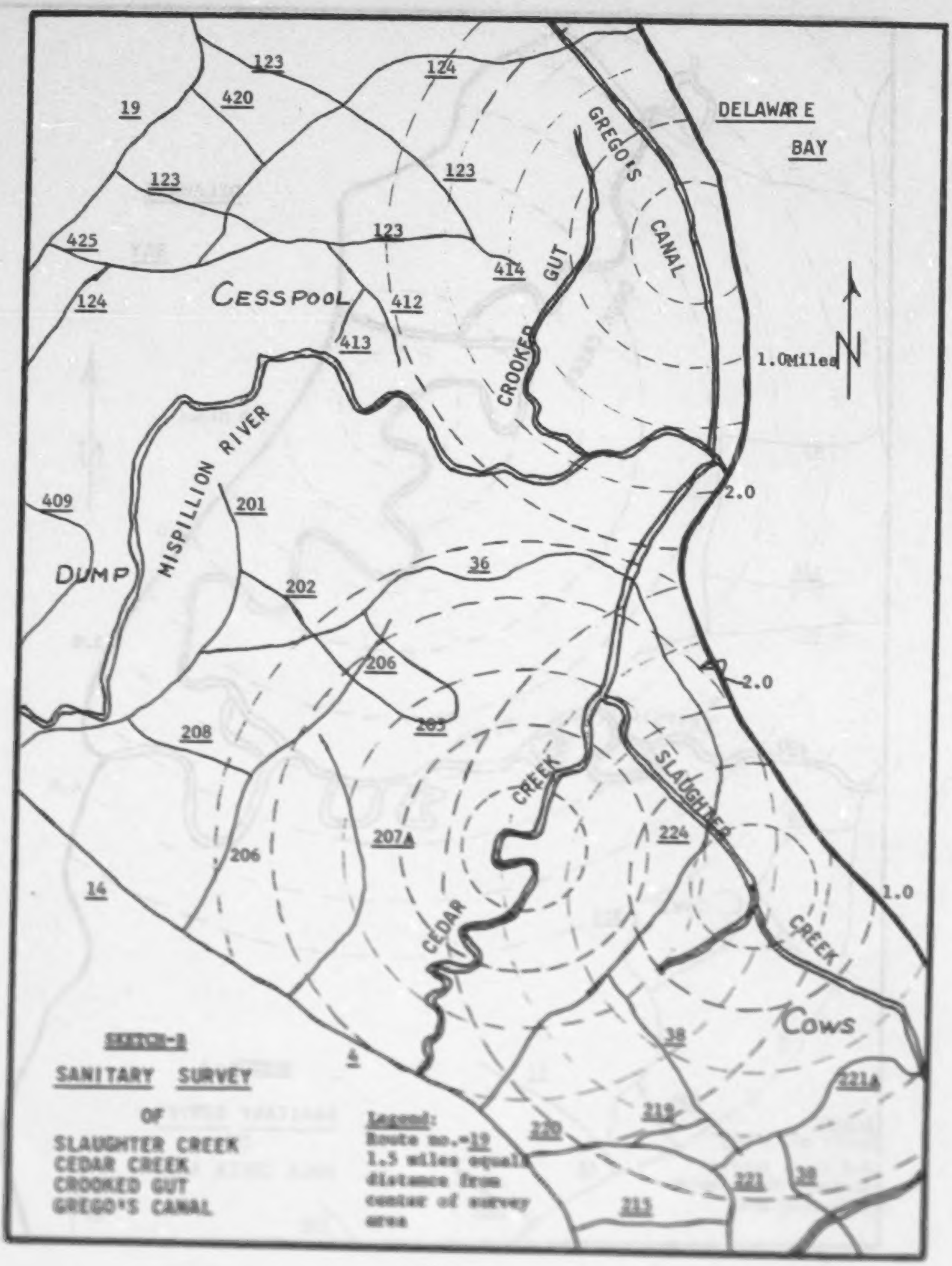
PENNA.
0
10
SCALE
N
A
NEW JERSEY
DELAWARE
DELAWARE
BAY
MARYLAND
B
C


DELAWARE
BAY
DUCK CREEK
LEIPSIC
RIVER
1.0 Miles
2.0
3.0
4.0
N
Cows
6
9
83
329
228
85
86
333
335
9
87
326
86
330
320
89

Legend:
Route no. = 206
2.0 miles equals distance from center of survey area

SKETCH - A

SANITARY SURVEY
OF
DUCK CREEK AREA



123
124
19
420
DELAWARE BAY
GREGO'S CANAL
123
123
425
414
124
CESSPOOL
412
413
CROOKED GUT
1.0 Miles
N
MISPILLION RIVER
2.0
409
201
DUMP
36
202
206
2.0
205
208
SLAUGHTER CREEK
CEDAR CREEK
207A
224
206
14
1.0
COWS
38
4
221A
219
220
221
38
215

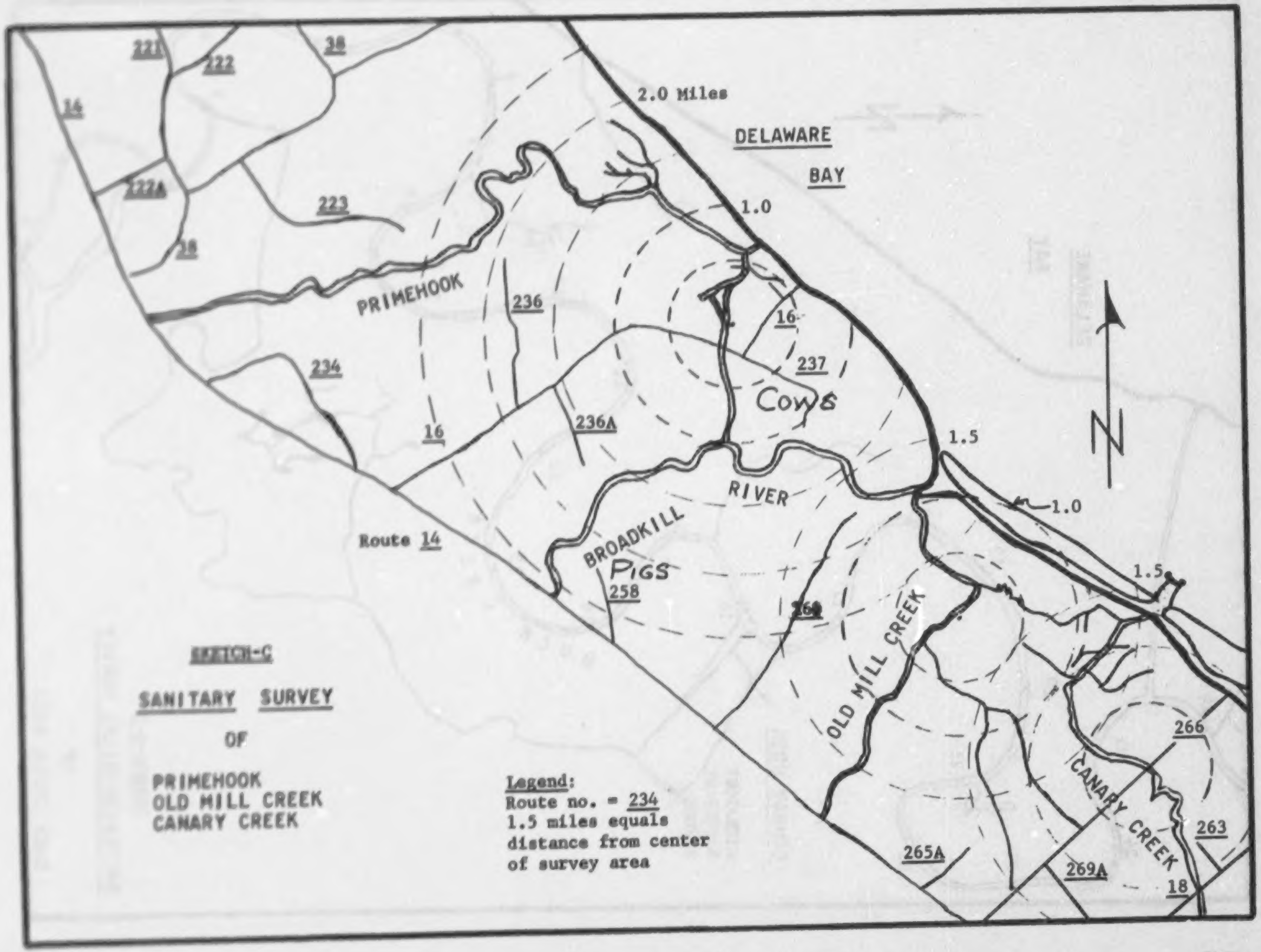
SKETCH-B

SANITARY SURVEY

OF

SLAUGHTER CREEK
CEDAR CREEK
CROOKED GUT
GREGO'S CANAL

Legend:
Route no.=19
1.5 miles equals distance from center of survey area

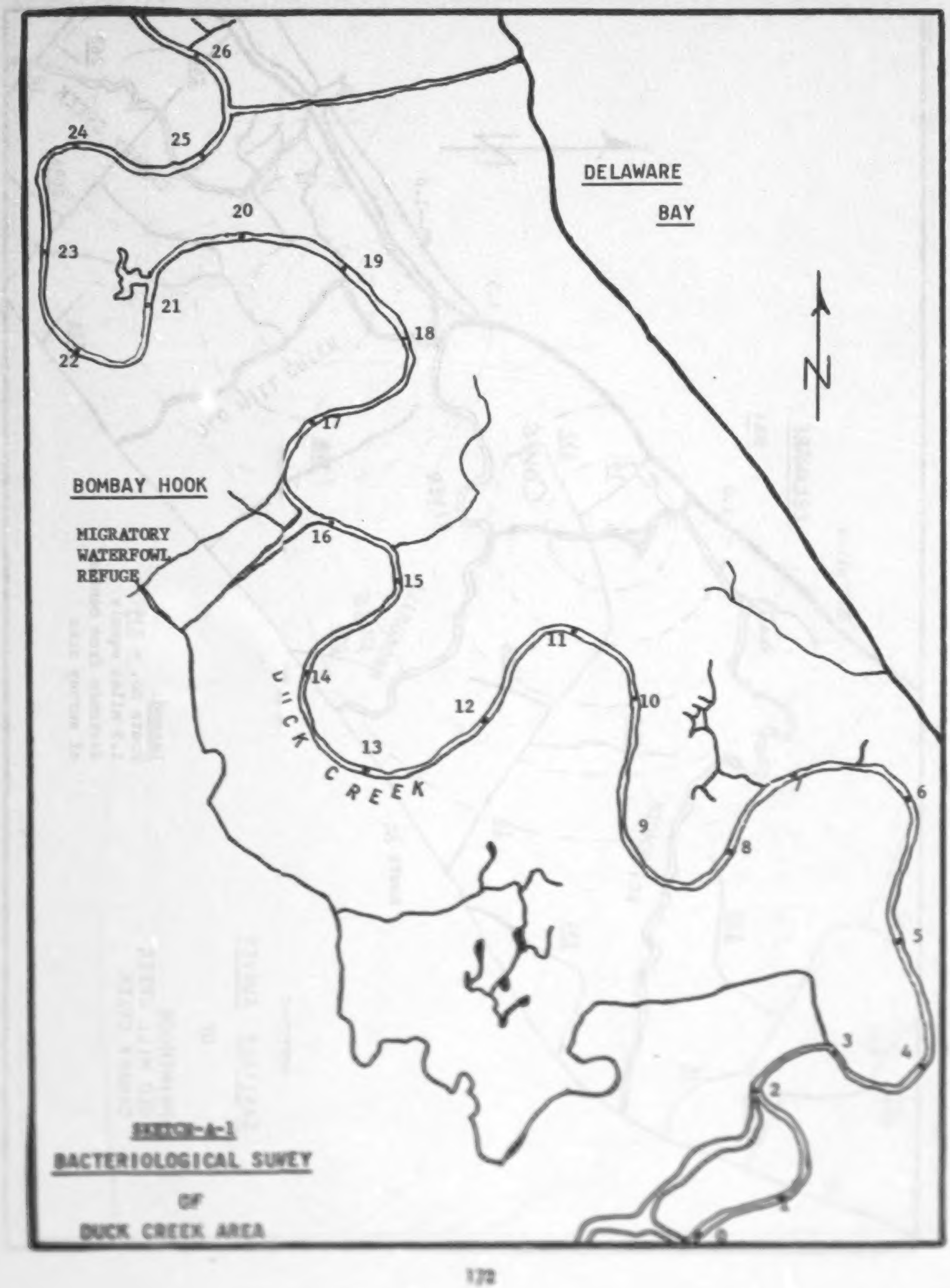


221
222
38
14
2.0 Miles
DELAWARE
BAY
222A
223
38
1.0
PRIMEHOOK
236
16
234
237
Cows
16
236A
1.5
RIVER
1.0
Route 14
BROADKILL
Pigs
258
264
1.5
OLD MILL CREEK
266
CANARY CREEK
263
265A
269A
18

SKETCH-C

SANITARY SURVEY
OF
PRIMEHOOK
OLD MILL CREEK
CANARY CREEK

Legend:
Route no. = 234
1.5 miles equals
distance from center
of survey area

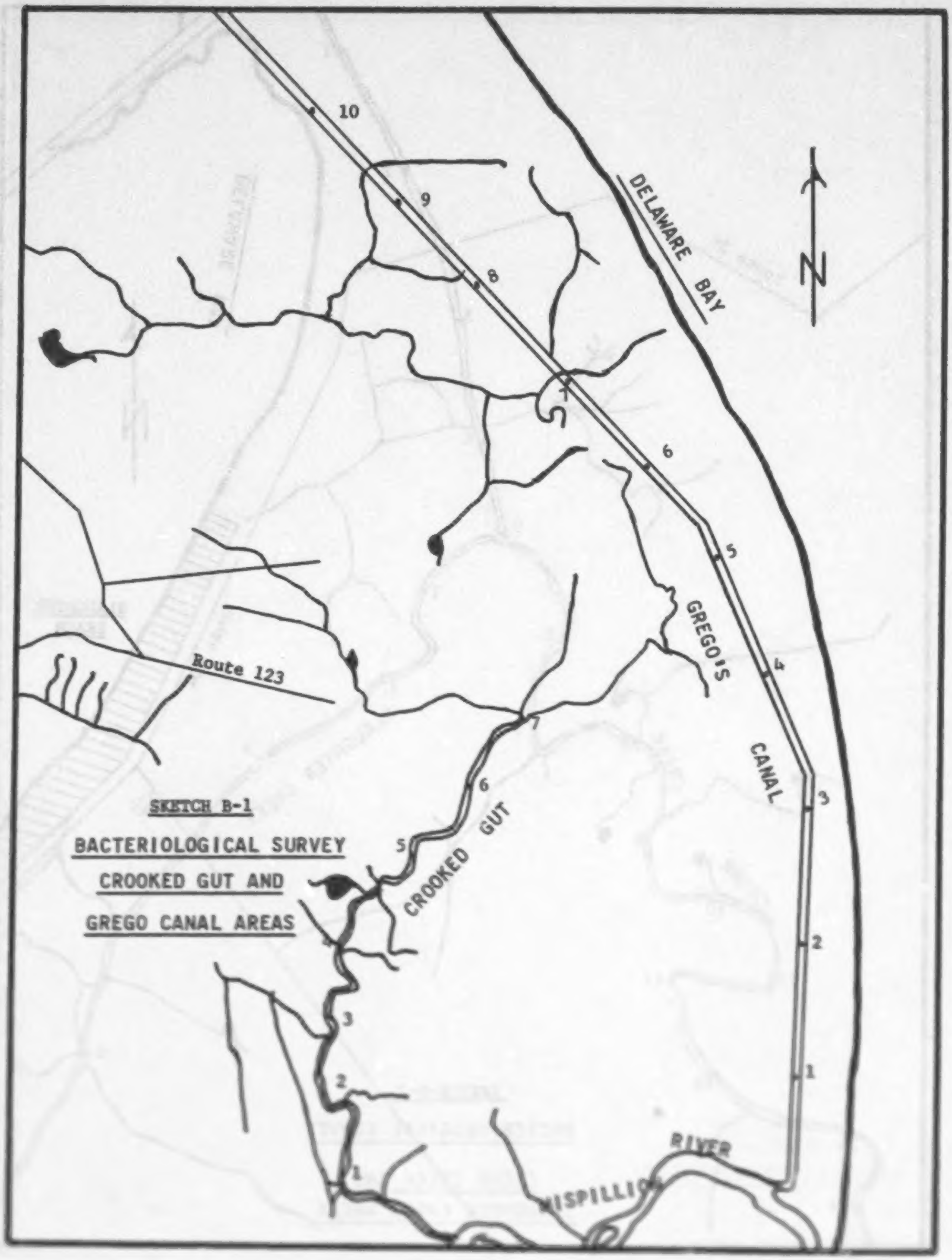

DELAWARE
BAY
BOMBAY HOOK
MIGRATORY
WATERFOWL
REFUGE
DUCK CREEK
N
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

SKETCH A-1

BACTERIOLOGICAL SURVEY

OF

DUCK CREEK AREA

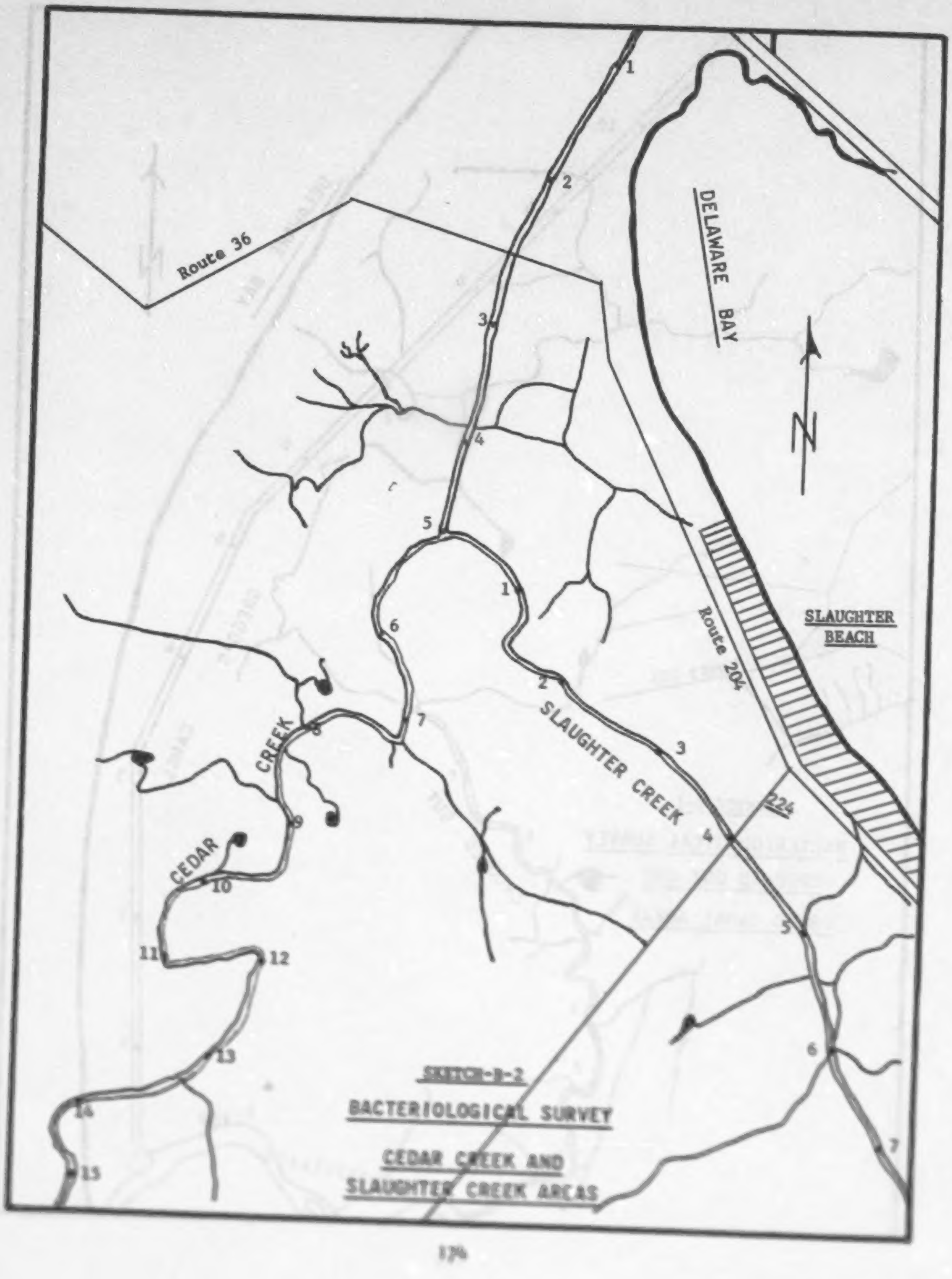

10
9
8
7
6
5
4
3
2
1
DELAWARE BAY
N
GREGO'S CANAL
Route 123
7
6
5
4
3
2
1
CROOKED GUT
MISPILLION RIVER

SKETCH B-1

BACTERIOLOGICAL SURVEY

CROOKED GUT AND

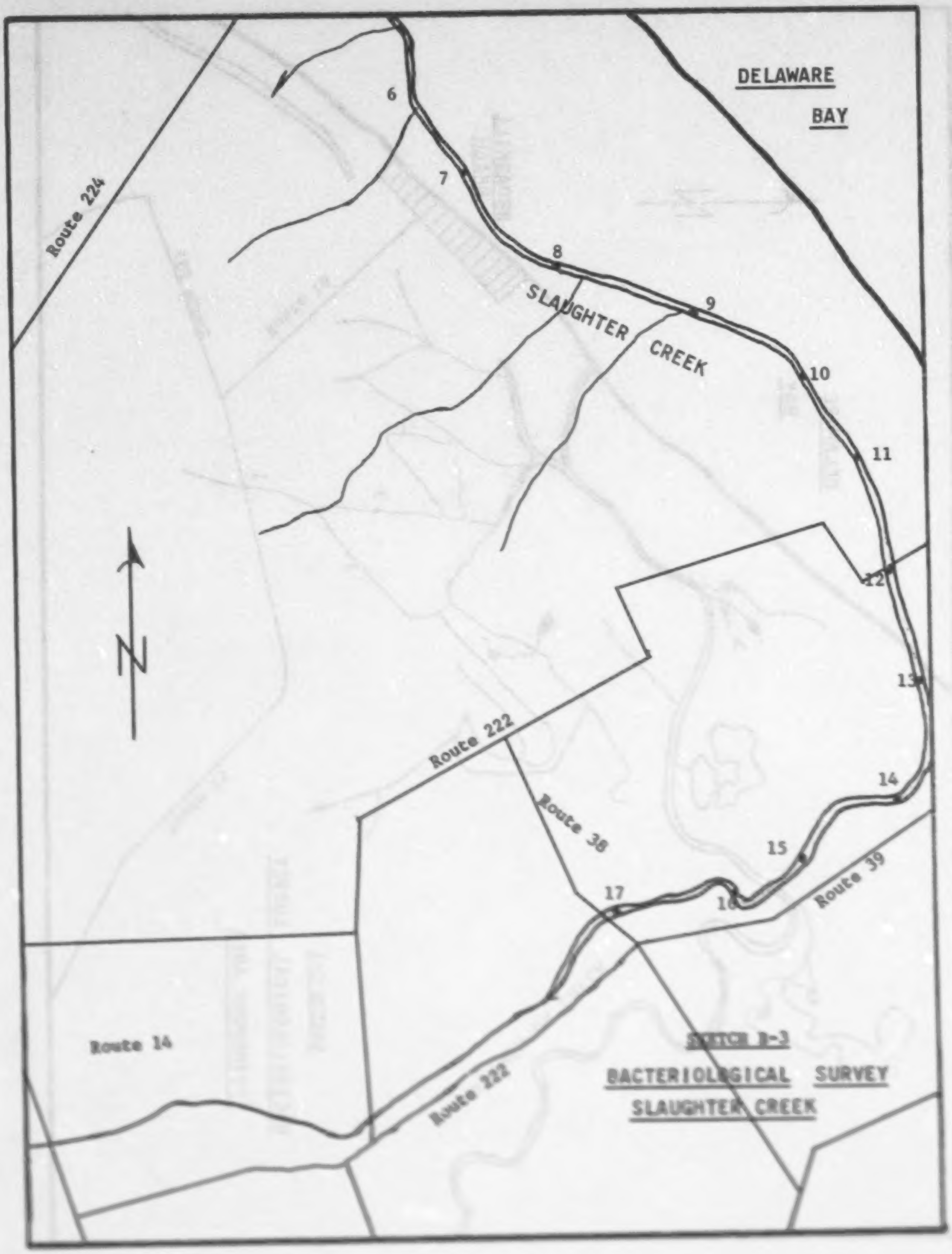
GREGO CANAL AREAS


1
2
Route 36
3
4
5
DELAWARE BAY
N
1
6
SLAUGHTER BEACH
Route 204
2
7
SLAUGHTER CREEK
3
8
CREEK
224
9
4
CEDAR
10
5
11
12
13
6
14
7
15

SKETCH-B-2

BACTERIOLOGICAL SURVEY

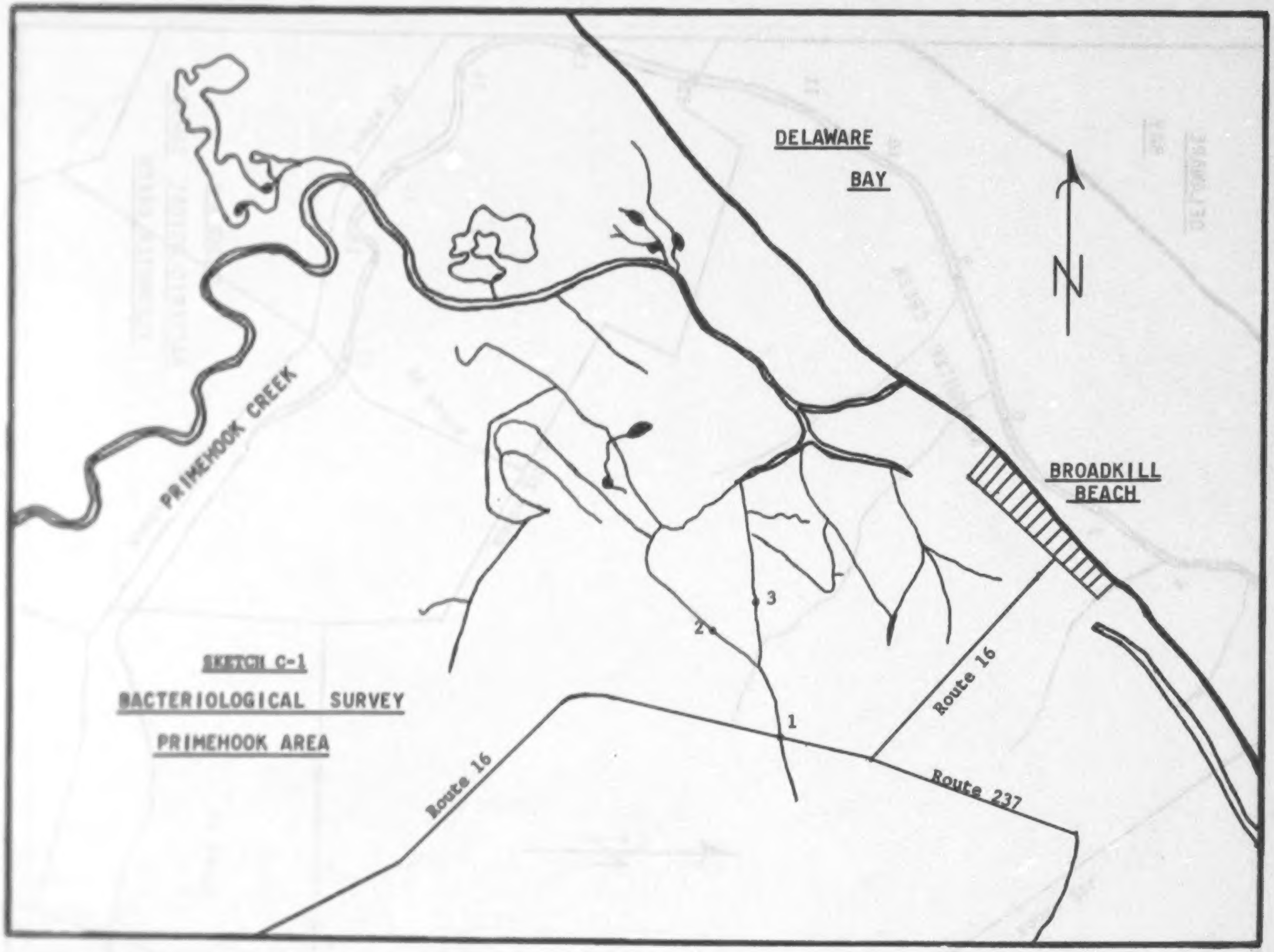
CEDAR CREEK AND
SLAUGHTER CREEK AREAS


DELAWARE
BAY
Route 224
6
7
8
9
10
11
12
13
14
15
16
17
SLAUGHTER CREEK
Route 222
Route 38
Route 39
Route 14
Route 222
N

SKETCH B-3

BACTERIOLOGICAL SURVEY

SLAUGHTER CREEK

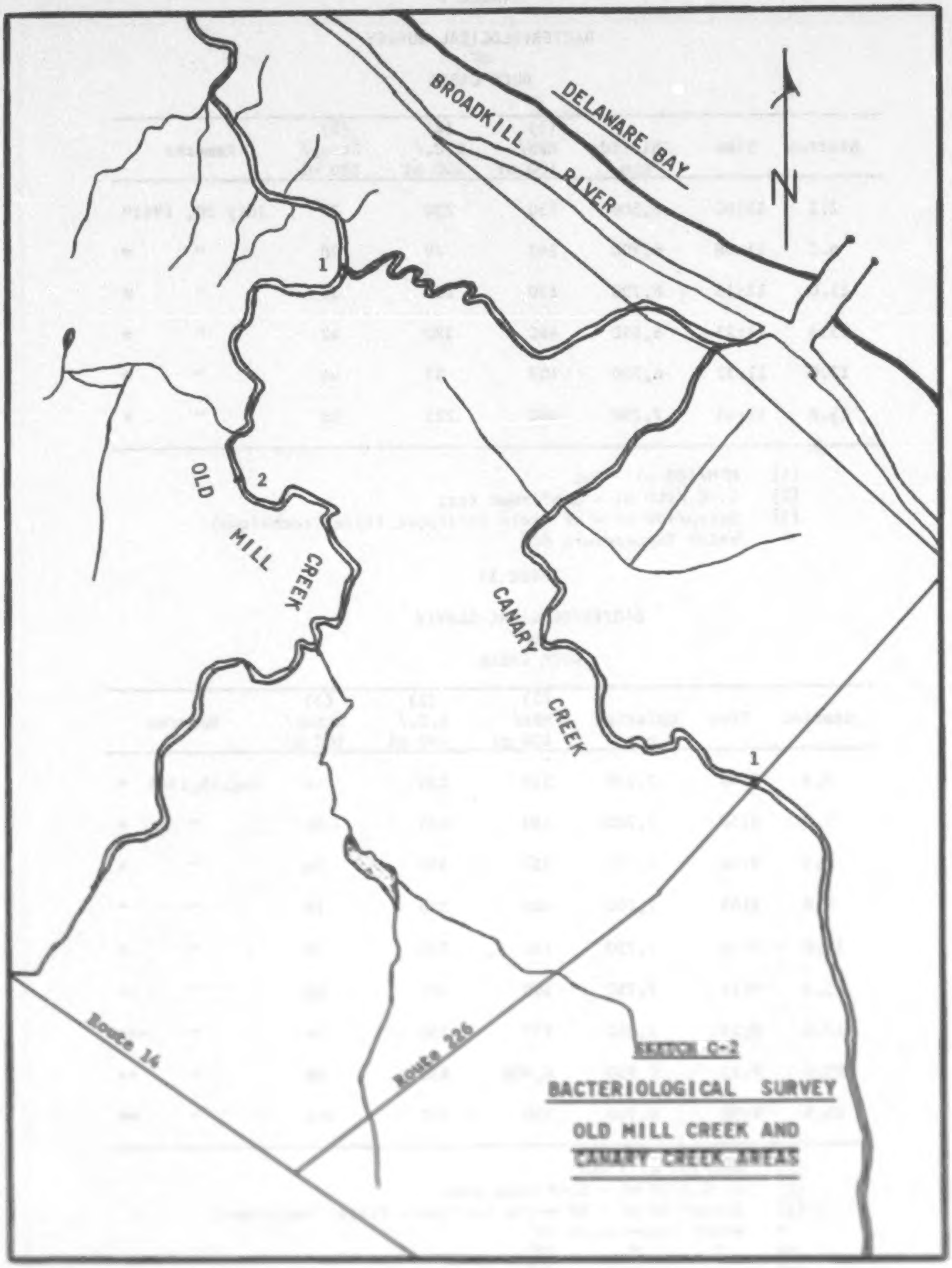


SKETCH C-1

BACTERIOLOGICAL SURVEY

PRIMEHOOK AREA

DELAWARE
BAY
N
BROADKILL
BEACH
PRIMEHOOK CREEK
1
2
3
Route 16
Route 237
Route 16


DELAWARE BAY
BROADKILL RIVER
N
1
OLD MILL CREEK
2
CANARY CREEK
1
Route 14
Route 226

SKETCH C-2

BACTERIOLOGICAL SURVEY

OLD MILL CREEK AND CANARY CREEK AREAS

TABLE I

BACTERIOLOGICAL SURVEY
OF
DUCK CREEK

Station	Time	Chloride ppm	(1) MPN/ 100 ml	(2) E.C./ 100 ml	(3) Strep/ 100 ml	Remarks
2.2	11:00	6,500	230	230	22	July 20, 1961*
6.5	11:08	6,700	141	79	28	" *
11.0	11:15	6,700	130	130	38	" *
15.5	11:27	6,650	460	330	42	" *
17.0	11:32	6,700	109	33	44	" *
23.0	11:45	7,250	490	221	46	" *

(1) MPN/100 ml - BBG
(2) E. C./100 ml - Confirmed test
(3) Strep/100 ml - KF media (milipore filter technique)
* Water Temperature 80°

TABLE II

BACTERIOLOGICAL SURVEY
OF
DUCK CREEK

Station	Time	Chloride ppm	(1) MPN/ 100 ml	(2) E.C./ 100 ml	(3) Strep/ 100 ml	Remarks
0.5	8:48	7,250	330	230	34	Aug.15,1961 *
1.0	8:50	7,700	330	330	20	" *
6.5	9:00	7,700	330	330	24	" *
9.0	9:05	7,700	490	330	16	" *
11.0	9:10	7,750	330	330	30	" *
15.5	9:15	7,750	490	490	80	" **
17.0	9:18	7,350	230	230	36	" ***
23.0	9:25	6,950	4,900	430	86	" **
25.5	9:30	6,550	330	330	162	" **

(1) MPN/100 ml - BBG
(2) E. C./100 ml - Confirmed test
(3) Strep/100 ml - KF media (millipore filter technique)
* Water Temperature 76°
** " " 73°
*** " " 75°

TABLE 3

BACTERIOLOGICAL SURVEY
OF
DUCK CREEK

Station	Chloride ppm	(1) MPN/ 100 ml	(2) Strep/ 100 ml	Remarks
2.2	6,000	790		July 17, 1961
23.0	7,350	2,400	64	Aug. 7, 1961
17.0	7,650	230	64	"
15.5	7,650	330	80	"
11.0	7,900	490	60	"
6.5	8,400	330	80	"
2.2	8,400	230	40	"
2.2	7,700	330	20	Aug. 15, 1961
2.2	7,650	4,900	60	Aug. 21, 1961

(1) MPN/DO ml - BBG

(2) Strep/100 ml - KF media (milipore filter technique)

TABLE 4

BACTERIOLOGICAL SURVEY
OF
CROOKED GUT

Station	Time	(1) MPN/100 ml	(2) Strep/100 ml	Remarks
1.5	3:40	790	80	Sept. 7, 1961
3.0	3:30	490	20	"
4.0	3:15	1,410	100	"
6.0	3:05	1,720	315	"
1.5	2:33	2,210	-	Sept. 5, 1961 *
3.0	2:37	3,100	50	" *
4.0	2:42	1,090	300	" *
1.0	-	900	116	Sept. 12, 1961

(1) MPN/100 ml - BBG

(2) Strep/100 ml - KF media (milipore filter technique)

* Water Temperature 30°

TABLE 5

BACTERIOLOGICAL SURVEY
OF
GREGO'S CANAL

Station	Time	(1) MPN/100ml	(2) Strep/100ml	Remarks
1.0	-	2,300	-	Sept. 12,1961
2.0	3:50	1,300	125	Sept. 7,1961
2.0	2:12	221	40	Sept. 5,1961*
4.0	2:18	2,300	120	Sept. 5,1961*
6.0	11:10	4,900	684	Sept. 14,1961**

(1) MPN/100ml - BBG

(2) Strep/100ml - KF media (milipore Filter technique)

* Water Temperature 30°
** Chloride - 11,000 ppm

TABLE 6

BACTERIOLOGICAL SURVEY
OF
CEDAR CREEK

Station	Time	(1) MPN/100 ml	(2) Strep/100 ml	Remarks
1	-	500	216	Sept. 12, 1961
2	-	1,700	196	"
2.5	3:00	3,300	170	Sept. 5, 1961*
3.0	-	2,200	180	Sept. 12, 1961
4.0	-	1,700	216	"
5.0	3:07	1,720	140	Sept. 5, 1961**
9.0	3:17	7,000	220	"
15.0	4:00	1,600	60	Sept. 8, 1961
2.5	3:40	580	92	"
15.0	3:23	7,000	220	Sept. 5, 1961***
12.0	3:20	10,900	172	Sept. 8, 1961

(1) MPN/100 ml - BBG

(2) Strep/100 ml - KF media (milipore filter technique)

* Water temperature 29°
** Water temperature 29.5°
*** Water temperature 30°

TABLE 7

BACTERIOLOGICAL SURVEY
OF
SLAUGHTER CREEK

Station	Time	Chloride ppm	(1) MPN/100 ml	(2) Strep/100 ml	Remarks
1.5	3:42	-	4,900	132	Sept. 5, 1961*
3.8	3:45	-	4,600	88	Sept. 5, 1961*
6.0	3:51	-	7,000	96	Sept. 5, 1961*
4.0	3:25	-	1,410	88	Sept. 8, 1961**
17.0	3:10	-	240,000	TNTC	Sept. 8, 1961**
1.0	-	-	200	96	Sept.12, 1961
2.0	-	-	1,300	76	Sept.12, 1961
17.0	-	425	240,000	45	Sept. 14,1961
4.0	10:00	2,750	4,600	22	Sept.14, 1961
12.0	-	750	240,000	81	Sept.14, 1961
14.0	3:00	-	13,000	40	Sept. 8, 1961

(1) MPN/100 ml - BBG

(2) Strep/100 ml - KF media (milipore filter technique)

* Water temperature 30.5°
** Air temperature 84°
Tide LWS (ebbing)

TABLE 8

BACTERIOLOGICAL SURVEY
OF
PRIMEHOOK CREEK

Station	Time	(1) MPN/100 ml	(2) Strep/100 ml	Remarks
1	2:30	4,900	116	Sept. 8, 1961*
1	9:00	9,400	47	Sept. 14, 1961**
2	1:50	13,000	TNTC	Sept. 6, 1961***
3	1:45	7,000	88	Sept. 6, 1961****

(1) MPN/100 ml - BBG

(2) Strep/100 ml - KF media (milipore filter technique)

* Air temperature 84°

** Air temperature 80° and chloride of 50 ppm

*** Water temperature 31°

**** Water temperature 30°

TABLE 9

BACTERIOLOGICAL SURVEY
OF
CANARY AND OLD MILL CREEKS

Station	Time	(1) MPN/100 ml	(2) Strep/100 ml	Remarks
		CANARY CREEK		
1	1:55	22,100	TNTC	Sept. 8, 1961
1	8:30	24,000	574	Sept. 14,1961*
1	-	4,600	712	Sept. 6, 1961
		OLD MILL CREEK		
1	-	790	234	Sept. 6, 1961
2	-	1,300	44	Sept. 6, 1961
1	-	700	124	Sept. 13,1961
2	-	3,300	84	Sept. 13,1961

(1) MPN/100 ml - BBG
(2) Strep/100 ml - KF media (milipore filter technique)

* Air temperature 80°
Sample depth 1.0'
Ebbing to slack water

Chloride - 3,400 ppm

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2. Interstate Commission on the Delaware River, Delaware State Water Pollution Commission, New Jersey Department of Health, Pennslyvania Department of Health, Water Department, City of Philadelphia, U. S. Public Health Service, Region II, "Dispersion Studies on the Delaware River Model Estuary", and "Potential Applications Towards Stream Purification Capacity Evaluations", June 1961.

3. Kaplovsky, A. J., "A Study of the Accumulation and Distribution of Iron Waste Discharges Within the Delaware River Attributed to Discharges Originating Within the State of Delaware", Dec. 5, 1952.

4. Kaplovsky, A. J., "Estuarine Pollution Investigations Employing Same-Slack Technique", Sewage & Industrial Wastes, Vol. 29, No. 9, Nov. 1957.

5. Kaplovsky, A. J., "Estuary Model Tests Expedite Prototype Studies of Pollution", Purdue Industrial Wastes Conference, May, 1961. (In print)

6. Kaplovsky, A. J., "Investigation of Sanitary Water Quality Within the Delaware River", Water Pollution Commission, State of Delaware Technical Report II, Dec. 1956.

7. Kenner, Bernard A., Clark, Harold F., and Kabler, Paul W., "Fecal Streptococci. II. Quantification of Streptococci in Feces", American Journal of Public Health, Vol. 15, No. 10, 1960.

APPENDIX M

RADIONUCLIDES IN THE MARINE ENVIRONMENT

By

J. E. Campbell
Milk and Food Research
Robert A. Taft Sanitary Engineering Center
Public Health Service
Department of Health, Education, and Welfare
Cincinnati, Ohio

I have been asked to discuss the occurrence and significance of radionuclides in the marine environment. Although this has been a topic of interest since about 1945 (corresponding to the first nuclear explosion), discussions at this time are particularly relevant in view of the recent resumption of weapons tests.

The sea is one of man's great natural resources and serves him in many ways. Probably its most important function is as a storehouse of food and, in view of the increasing world population, this function will become increasingly important.

Since most commercially important marine organisms are harvested in coastal waters or in offshore waters near land, pollution of the sea is of real concern lest we create a situation that will curtail our use of this important natural resource.

Marine animals are ultimately dependent upon the photosynthetic activity of green plants, which are their primary source of energy. The food chains from these plants to the various species of marine organisms are varied and complex.

Radioactive substances in the marine environment come from a number of sources. Other than the naturally occurring radioisotopes such as K-40, the most important source at the present time probably is the fallout from nuclear weapons tests. Other sources include waste discharges from power or other types of peace-time reactors and nuclear vessels, sewage effluents from hospitals and industry, and sea disposal of radioactive wastes. Disposal of all of these radioactive wastes appears to be well controlled and in my opinion does not constitute any real problem at this time.

In a typical nuclear detonation between 150 and 170 fission products are formed; collectively these are considered "fallout." Because of very short half-life or extreme insolubility, most of these products are of little or no biological significance. Those generally regarded to be of concern are iodine-131, strontium-90, strontium-89, cesium-137, and barium-140. In addition to the true fission products other activation products also may be formed under certain test conditions, through neutron bombardment of stable parent elements. Included in this group of radionuclides are cobalt-60, iron-55, iron-59, iron-65, and zinc-65. When a nuclear detonation occurs, these fission and activation products are forced high into the atmosphere and subsequently return to earth as dust or with rain. Radionuclides deposited in the ocean rapidly disappear from the water through uptake by bottom materials and biota. This uptake is so great that, even with repeated doses of fallout, radionuclide concentrations in the water remain very low.

With one or two exceptions, the accumulation of radionuclides by the higher marine forms comes from ingestion of food and not directly from the water.

The primary food source, the phytoplankton, is noteworthy for its ability to concentrate most radionuclides and trace elements. How this is accomplished is not known, although it has been shown that surface absorption is involved; however, the phytoplankton does provide a concentrated source of these materials to organisms such as the zooplankton, which feeds on them. These minute animal forms are filter feeders and in turn serve as an important source of food for small fishes. The zooplankton represents a large concentration of radionuclides and trace elements acquired from the materials taken in with the phytoplanton and directly from the water.

Shellfish also are filter feeders that concentrate trace elements and radionuclides. For example, we have examined some oysters that contained up to 3.5 to 4.0 percent zinc on a dry weight basis, even though it is unlikely that the waters from which these shellfish were taken contained over 0.02 ppm.

In general strontium-90 is accumulated only by organisms that have calciferous structures. Essentially no strontium-90 is concentrated from water by the phytoplankton or by most zooplankton. Thus the food chain does not provide an ever-increasing concentration of this radionuclude in the more complex marine forms. Although radiostrontium is taken up from water by shellfish, it is concentrated almost exclusively in the shell. The following table shows the strontium-90 content of the edible portion of clam and oyster samples taken during the early part of 1960. The levels of strontium-90 are very low in comparison to the levels in most landderived foods assayed at the same time.

SHELLFISH ANALYSES

Code Location	A	B	C	D	E	F	G	H
Sr^{90}, μμc/kg	1.3	-	0.91	1.1	1.6	1.4	2.0	0.71

On the other hand oysters and clams may contain relatively high levels of zinc-65, cobalt-60, and iron-59, but from a health standpoint, these materials present essentially no problem. Cesium-137 is also accumulated to relatively high levels in the soft tissues of the shellfish, but as with the radionuclides mentioned above, the cesium-137 does not present a significant health problem at the levels found.

Because of the high concentrations of stable iodine in sea water, the resulting isotopic dilution makes it unlikely that shellfish would accumulate high concentrations of iodine-131. In addition this radionuclide has only an 8-day half-life and much of any that was incorporated into shellfish tissue would decay between the time of harvesting and eating.

We have seen that the sea is influenced by the radionuclides that are deposited therein Through various food chains some of these materials end up in edible species of marine life in appreciable concentrations. In the event of serious contamination of the land masses of the earth, the sea will undoubtedly be a great source of food for man. For this reason, we must carefully consider all matters relating to the contamination of the sea by radioactive substances, whether this contamination results from waste disposal practices or from other sources.

APPENDIX N

RESULTS OF INTERSTATE COLLABORATIVE STUDY TO EVALUATE INTERIM BACTERIOLOGICAL STANDARDS FOR SHUCKED OYSTERS

By

C. B. Kelly
Public Health Service
U. S. Department of Health, Education, and Welfare

In 1956, interim bacteriological standards were adopted at the Shellfish Sanitation Workshop to be applied to shucked oysters as received at the market. The limits adopted at that meeting are given below.

> "Class 1. Acceptable: Shucked oysters with a Most Probable Number (MPN) of coliform bacteria of not more than 16,000 per 100 ml., and/or a Standard Plate Count of not more than 50,000 per ml.
>
> Class 2. Acceptable on Condition: Shucked oysters with a coliform MPN greater than 16,000 per 100 ml., but less than 160,000 per 100 ml., and/or a Standard Plate Count greater than 50,000 per ml., but less than 1,000,000 per ml. (The oysters will be accepted on the condition that the shellfish sanitation authority in the originating State will make immediate investigation of the producer's plant and operations and will submit a report of such investigations to the control agency in the market area. On the basis of this report the control agency in the market will reject or permit further shipments from the producer in question.)
>
> Class 3. Rejectable: Shucked oysters with a coliform MPN of 160,000 or more per 100 ml., and/or a Standard Plate Count of 1,000,000 or more per ml."

At the 1956 Workshop doubt was expressed as to the adequacy of at least the coliform limits. The change in the acceptable coliform limit from MPN 2400 to MPN 16,000 reflected the attitude of the group on the significance of coliform organisms. This change met with objection from northern interests, and rightfully so, becuase coliform levels of that magnitude in oysters harvested or processed in northern areas would quite likely indicate serious pollution or contamination.

Data were presented which demonstrated a high coliform organism count in shell and shucked oysters in the face of evidence that these oysters were from safe, approved areas and had been processed in approved plants in conformity with the requirements of the Public Health Service Manuals. Thus, the coliform group failed in its prime purpose which was to reveal whether the shellfish had been harvested from polluted areas or had been exposed to serious contamination during handling and processing after removal from the water.

The group therefore recommended that an evaluation of the interim standards be made, and that serious study be given to the validity of the coliform group as an index of quality; it further recommended that simultaneous investigations be conducted to evaluate other bacterial indices. E. coli was suggested as a possible substitute for the entire coliform group.

The evaluation of the 1956 standards and of the suitability of E. coli was made collaboratively during the oyster seasons in 1956, 1957 and 1958. The data were collected by the collaborating agencies which met and discussed them on June 26, 1958; and their findings, with recommendations, were presented at the 1958 Shellfish Sanitation Workshop.

Based on the conclusions and recommendations of the report of this group the Workshop recommended the following:

1. The coliform group of organisms is not a meaningful indicator of pollution in shucked oysters at the market. Substitution of the fecal coliform organisms (those coliform organisms capable of growth with the production of gas in E C medium at elevated incubation temperatures according to procedures established in the 1956-58 cooperative study) was recommended.

2. The plate count should be included as an index of contamination during processing and to detect faulty handling or refrigeration practices during transportation and marketing.

3. The sanitary significance of fecal coliform organisms cannot be established properly without reliable information on their origin and introduction into shellfish growing areas. It was recommended therefore, that studies be conducted in shellfish growing areas to determine the significance of these organisms and that the Public Health Service be requested to develop a uniform procedure to be followed by the States in conducting sanitary surveys in areas of known pollution. Preliminary results of these studies were discussed at the 1961 Shellfish Sanitation Workshop.

The following procedures and bacteriological standards for shucked Eastern oysters at the market level were recommended at the 1958 Workshop for use in the Cooperative Program for the Certification of Interstate Shellfish Shippers during 1959 and 1960:

Satisfactory:

A fecal coliform density (MPN) of not more than 78 per 100 ml. of sample, and a plate count of not more than 100,000 per ml. of sample; in occasional samples fecal coliform density up to and including 230 per 100 ml. of sample and/or a plate count up to and including 500,000 per ml. of sample will be acceptable. (For convenience, these will be referred to as Class I-A and I-B samples, respectively.)

The official agency in the receiving area should notify both the shipper and the shellfish control agency at the point of production of any Class I-B samples. If two consecutive Class I-B shipments are found, the official agency in the receiving area is justified in excluding the shipper from the market until a satisfactory report on the shipper is received from the state control agency in the producing area.

Unsatisfactory:

A fecal coliform density (MPN) of more than 230 per 100 ml. of sample or a plate count of more than 500,000 per ml. of sample. (Class II samples.)

The official agency in the receiving area should immediately notify both the shipper and shellfish control agency in the producing area of a Class II sample. A single Class II sample is justification for excluding the shipper from the market pending receipt of a satisfactory explanation from the official control agency in the producing area.

> As used in the interim bacteriological standard, "fecal coliform organisms" are those organisms which, on transfer to an E C medium from gas positive presumptive broth tubes, show the production of gas after incubation in a water bath at 44.5°C for 48 hours.

The procedure described in the Appendix recognizes air incubation at 45.5°C ± 0.2°C as a possible alternate to incubation in a water bath at 44.5°C ± 0.2°C. Air incubation is acceptable only if a uniform lag period and temperature control are attained throughout the incubator under all conditions of loading.

To implement the recommendations of the 1958 Workshop, a conference was held at Virginia Beach in October 1958, to develop a plan for evaluating the interim bacteriological standards. The plan decided upon involved the collection and examination of oysters at strategic points of harvesting, shucking, and repacking and as received in the marketing areas.

Tests were conducted for fecal coliform organisms, coliform organisms, and plate count, according to the procedures recommended by the 1956-58 collaborative study group at the 1958 Workshop. (The recommendation to evaluate the sanitary significance of these organisms in shellfish areas has been carried out in another study).

RESULTS

As a result of the planning conferences the agencies participating in the collaborative study requested the Public Health Service to act as the central agency for co-ordinating the activities of the study by compiling the data collected and preparing statistical summaries of the results. All the participating agencies, therefore, sent their data to the Shellfish Sanitation Laboratory.

This report presents a statistical summary of the data collected, some comparisons of the old coliform index with the new fecal coliform organisms, and some observations on conformance with the revised plate count standards as well as with the new tolerance for fecal coliforms.

The types and origin of the samples examined by the participating agencies are presented in Table 1. A total of 2,022 samples were examined. The various figures summarize results from the fall of 1958 to April, 1960.

Summarization of the Data

The results obtained on the samples were plotted on logarithmic probability paper in accordance with the method described by Velz and later by Thomas. This plotting of the data provides a simple and efficient method of analysis. In one manipulation one obtains the geometric mean and the frequency of occurrence at any desired percentile, and by extension of the line within limits one is able to predict the occurrence at any desired value or the value at any desired percentile occurrence. The plot fundamentally tests the extent to which the data are normally distributed and the standard deviation or variability of the data. Plotting two sets of data on the same graph reveals the relationship between these two sets of data. For example, the coliform MPN's and the E C gas positive MPN's were plotted on the same graph to show their relationship.

Plots were prepared of the data as grouped by season, primarily to discover possible seasonal differences; no plots were prepared for the combined results of all seasons, (all samples). The samples collected by the various states were also plotted separately. Plots were not prepared of combined results in each sample category (shellstock, as packed, as repacked).

Figures 1, 2, 3 and 4 illustrate the type of plots obtained and are representative of the entire group. All of the plots show a high degree of variability, as indicated by the steepness of the line. To illustrate the variability of the data, the 95% confidence limit of the MPN limit of enumeration is shown on the plots as dotted lines. Regardless of the high degree of variability there was almost always an exceedingly high conformity of the individual points with the line of best fit, indicating that the data conformed with the accepted line of normal distribution.

The geometric means, together with the values at 5, 10, 50, 90 and 95 percent occurrence are presented in Tables 2, 3 and 4.

Results Obtained in Oyster Shucking Houses

As shown in Table 2, Virginia and Maryland examined approximately 200 samples each of shell oysters in shucking houses, almost equally divided between the two seasons. The mean coliform MPN during the winter months was determined to be 510 and 160; for the spring and fall months 3900 and 1500. Thus, the coliform MPN of 230 recommended in the manual is met in only one of the four series of samples. The median E C gas positive MPN of these same samples was found to be less than 18 during the colder months; 55 and 74 during the warmer months.

A comparison of the shell and shucked oyster results is of interest. In all cases an increase in coliform MPN occurred during the shucking process but a study of the E C gas positive results indicates a decrease during the shucking and washing process. The plate count results parallel those obtained for coliform organisms. Of interest also might be the similarity of the fecal coliform MPN's obtained in Virginia, Maryland, and New Jersey on shucked oysters during the winter months.

The relatively high E C gas positive MPN's of shell oysters at the 90 percentile level during the spring and fall months result in similarly high values in the shucked oysters. Multiplication of coliform organisms during this period is quite obvious from MPN's listed in Table 2, for both the mean and the 90 percentile values. The likelihood of multiplication of E C gas positive organisms in occasional shipments subjected to adverse conditions somewhere between harvesting and immediately before shucking should not be disregarded.

Samples Collected in Repacking Houses

Data on samples from repacking houses collected by Maryland and New Jersey are presented in Table 3. Maryland collected 170 samples of shucked oysters as received, almost equally divided between the two seasons. Approximately 60 repacked samples were collected by Maryland, again almost equally divided between the two season groups. Similar numbers of as-received and as-repacked oysters were collected by New Jersey during the winter season. However, fewer samples were collected by New Jersey during the warmer months.

The oysters as received in both Maryland and New Jersey packing houses are characterized by relatively high coliform MPN's. The mean coliform MPN of the product as received in the winter was 7,800 and 2,500 for Maryland and New Jersey respectively and in the spring and fall 15,000 and 28,000.

A comparison of these values with the average coliform MPN of shucked oysters as packed in shucking houses, would show considerable differences; the product as received at the repackers would be considerably higher in coliform density. For example, the mean coliform MPN of as-packed oysters in Maryland plants during the winter months was 880. This might be compared with 7,800 as received in repacking houses. A similar relationship can be seen by comparing the spring and fall samples which show a coliform MPN of 2900 as packed and 15,000 as received in the repacking plants. This is, of course, not a direct comparison, since these are not traced lots. However, the figures represent sizeable numbers of samples and represent attainment at least in the shucking houses and what has been observed from a sizeable number of samples in the repacking establishments.

Similar relationships can be seen in plate counts; the average plate count in warmer and colder periods of as-packed oysters in Maryland is 1,300 and 7,000 respectively as compared with a count of 26,000 and 6,800 in oysters as received in repacking houses.

Of particular concern is the relatively high E C gas positive MPN's obtained on the oysters as received at repacking houses. Although in three of the four sets of data presented in Table 3, the average E C gas positive MPN might be considered low (at least the medians fall below the acceptable MPN of 78 stipulated in the eastern standards), a few but significant number of samples show high E C gas positive MPN's. The coliform and plate count results are strongly suggestive of multiplication in transit, inasmuch as the as-packed samples in shucking houses in those states show lower results. Thus it is not unlikely that a simultaneous multiplication of fecal coliform organisms might take place under adverse conditions of refrigeration or during excessively long time periods between packing and delivery to the repacking establishments.

These high E C gas positive MPN values are real and are not the result of the method of summarization. For example, of the 82 samples as received in Maryland repacking houses during the winter, 22 were in excess of 78, 12 in excess of 230, and 2 in excess of 2,400; of the 88 samples as received during the summer, 38 were in excess of 78, 27 in excess of 230, and 6 higher than 2,400. New Jersey results show similar patterns.

Determination of the influence of the washing operations on the bacterial content of the oysters is difficult because of the unequal number of samples collected. The results indicate that there is usually a reduction in the coliform and plate count values.

Samples of Shucked Oysters Collected in the Market Areas

Data on samples obtained from the market area are presented in Table 4. Data were supplied by the Chicago Board of Health, the New York City Department of Health and the New York State Conservation Department. The New York City and New York State results are subdivided into southern and northern shippers, southern being from the states south of New Jersey, northern from New Jersey and the other New England states. Inclusion of New Jersey as a northern source might be questioned because a large part of the oysters from that state, particularly those from repacking plants, originated in Maryland and Virginia. The data supplied by the agencies in the market area, however, showed large numbers as having their source in Delaware Bay and thus New Jersey was included with the northern producing states.

New York City and New York State examined similar numbers of samples, with seasonal and geographical distribution also quite similar (Table 4). The Chicago Board of Health examined approximately 150 samples, with sources so diversified that sub grouping was not possible. The Chicago Board of Health did not run tests for coliform organisms.

The coliform results in practically all of the sample categories show poor compliance with the 1956 interim standard of 16,000 MPN; they also show distinct seasonal differences. The E C gas positive MPN's in all season source groups but one showed good compliance with MPN 78. During the warm months, however, there appears a small but significant number of samples that show high fecal coliform values. The highest value at the 90 percentile was from the data supplied by the Chicago Board of Health, the point most distant from the source of the product. MPN 2200 at the 90 percentile obtained from the samples of northern oysters tested by New York State may not be real because of the small numbers of samples examined.

Again one might speculate as to whether the presence of these high E C gas positive MPN's at low frequencies of occurrence might be due to some adverse treatment during shipment. The fact that 50% of the samples showed such low values is some evidence of such a possibility.

Chicago shows also the highest plate count results; the seasonal variation is also evident from the values derived from the data from all agencies.

To show attainment of the limits recommended in the tentative market criteria, the data were assembled in a different form. Tables 5, 6, and 7 show the percent attainment of coliform, E C gas positive, and plate count limits. Although the market criteria do not apply at present to samples taken in the production area, values for as-packed and as-repacked oysters are included in the tables.

Table 5 shows that compliance with coliform MPN 16,000 was reached in a relatively low percent of the samples collected in the warmer months. Even during the winter months only approximately three-quarters of the samples complied as examined by New York City; however, 92% complied as examined by New York State. A similar seasonal pattern was found by both agencies; particularly in the warmer months compliance generally was low.

By comparison, much higher percent attainment of the E C gas positive MPN's 78 and 230 was found by all agencies. Again this shows the seasonal pattern of difference. Between 82 and 90 percent of the samples collected in the marketing areas during the winter showed MPN's of 78 or less and between 91 and 96 percent showed 230 or less. New York City found the lowest attainment of MPN 78 during the warmer months, 43 percent of which was in 19 samples of oysters from northern sources. The attainment by other agencies and from other areas varied from 43 percent to 75 percent. MPN 230 was attained in 61 percent of the samples twice, 76, 80 and 84 percent in other sample groups.

The plate count values in samples examined by all agencies are presented in Table 7. Attainment of plate counts 5,000, 10,000 and 50,000 are presented primarily to show the pattern of distribution in the various sample categories and seasons. Of particular interest, of course, would be attainment of 100,000, 500,000, and 1,000,000.

Again the lowest conformity with any of these values was in the samples collected by the Chicago Board of Health, which also had a low degree of seasonal variation, if any. The plate counts during the warmer months showed higher attainment than those collected during the winter months. This might be explained by a review of the shipping practices.

In all sample groups except those of Chicago there appeared a high compliance with plate count 100,000. During the colder months, a compliance of 83 to 91 percent was found by New York City and New York State and during the warmer months between 75 and 86 percent. Of interest also is the very high compliance with plate count 500,000. All samples except those in one group showed compliance in excess of 90 percent. Practically all showed compliance with the 1,000,000 plate count value.

SUMMARY

As stated, the purpose of this report is primarily to assemble and summarize the data collected by the collaborating agencies and to conduct such statistical analyses that would reveal the changes in densities of the bacterial indices in question at the various levels of production to allow the association of such changes with observed conditions in commercial practices.

The collaborating agencies have produced data of extreme value in assessing the applicability of the 1958 market criteria. With very few exceptions the determined values in the various sample groups showed a pattern of normal distribution, which indicates that during the period of the study and in the areas studied, the data collected are representative.

The data on coliform tests appear to confirm the previous conclusions that this index is of not more value than the plate count in assessing the quality of the product or in the detection of adverse treatment. Coliform organisms and plate count appear to multiply in similar fashion as the product proceeds through the various commercial handling practices.

Tests for fecal coliform organisms show lower rates of change, although they do reflect changes that are expected during commercial handling processes. This is particularly true if mean values at the various levels of production are considered.

Throughout many of the sample groups there appear small, but significant frequencies of high fecal coliform densities. The fact that these occur at a frequency of 10 percent or less would suggest that they might have come as the result of adverse treatment during the particular stage of processing.

TABLE I

SAMPLES EXAMINED

	Number of Samples						
Agency	Shucking Houses		Repackers		Shucked Market Oysters		
	Shell	Shucked	as rec'd	repacked	Southern	Northern	All
Dec. - Jan. - Feb. - Mar.							
Virginia	112	114					226
Maryland	110	165	82	29			386
New Jersey	2	36	79	24			141
New York City					153	25	178
New York State					102	17	119
Chicago					83		83
All	224	315	161	53	338	42	1133
Apr. - Sept. - Oct. - Nov.							
Virginia	87	90					177
Maryland	100	160	88	27			375
New Jersey	8	2	28	3			41
New York City					91	19	110
New York State					93	14	107
Chicago					79		79
All	195	252	116	30	263	33	889
All Seasons							
Virginia	199	204					403
Maryland	210	325	170	56			761
New Jersey	10	38	107	27			182
New York City					244	44	288
New York State					195	31	226
Chicago					162		162
All	419	567	277	83	601	75	2022

TABLE II

COLIFORM AND E C GAS POSITIVE MPN'S AND PLATE COUNTS AT PROBABILITY PERCENTILES 5, 10, 50, 90 and 95

Shell and Shucked Oysters In Shucking Houses

Percen- tile	Coliform MPN						E C Gas Positive MPN						Plate Count					
	Shell oysters			Shucked oysters			Shell oysters			Shucked oysters			Shell oysters			Shucked oysters		
	Va.	Md.	N.J.	Va.	Md.	N.J.	Va.	Md.	N.J.	Va.	Md.	N.J.	Va.	Md.	N.J.	Va.	Md.	N.J.
	Dec. - Jan. - Feb. - Mar.																	
5	21	<18	--	230	16	<18	<18	<18	--	<18	<18	<18	--	--	--	230	--	280
10	43	<18	--	400	39	10	<16	<18	--	<18	<18	<18	--	--	--	450	--	430
50	510	160	--	3000	880	180	<18	<18	--	<18	<18	17	490	<3000	--	5000	1300	1800
90	6400	6700	--	22000	20000	3000	96	120	--	60	80	190	10000	2200	--	54000	20000	8000
95	13000	20000	--	39000	48000	6300	230	640	--	200	280	370	25000	5200	--	110000	43000	12000
*	112	110	2	114	165	36	112	109	2	114	165	36	109	104	2	112	163	36
	Apr. - Sept. - Oct. - Nov.																	
5	270	33	--	520	90	--	<18	<18	--	<18	<18	--	--	--	--	1400	270	--
10	490	78	--	890	230	--	<18	<18	--	<18	<18	--	350	--	--	2600	540	--
50	3900	1500	--	5800	2900	--	55	74	--	39	16	--	4900	2800	--	22000	7000	--
90	30000	29000	--	37000	37000	--	780	5500	--	700	1500	--	68000	82000	--	180000	88000	--
95	54000	68000	--	64000	78000	--	1600	19000	--	1600	5400	--	150000	210000	--	320000	180000	--
*	87	100	8	90	160	2	87	100	8	90	100	2	85	93	8	89	153	2

*/ Number of samples

TABLE III

COLIFORM AND E C GAS POSITIVE MPN'S AND PLATE COUNTS AT PROBABILITY PERCENTILES 5, 10, 50, 90 AND 95
Shucked Oysters at Repacking Houses

Percentile	Coliform MPN				E C Gas Positive MPN				Plate Count			
	As received		Repacked		As received		Repacked		As received		Repacked	
	Md.	N.J.	Md.	N.J.	Md.	N.J.	Md.	N.J.	Md.	N.J.	Md.	N.J.
					Dec. - Jan. - Feb. - Mar.							
5	290	55	410	<18	<18	<18	<18	<18	550	7000	--	460
10	700	130	720	<18	<18	<18	<18	<18	950	11000	470	870
50	7800	2500	5300	54	14	<18	<18	<18	6800	54000	6800	8700
90	84000	16000+	38000	2700	550	110	2400	29	49000	260000	62000	87000
95	160000	16000+	68000	8000	1600	290	--	170	84000	400000	140000	160000
No. of samples	82	79	29	24	82	79	29	24	80	79 (2)	29	23
					Apr. - Sept. - Oct. - Nov.							
5	1400	2700	1600	--	<18	<18	<18	--	1800	29000	2500	--
10	2400	4600	2700	--	<18	<18	<18	--	3300	43000	4200	--
50	15000	28000	20000	--	70	450	72	--	26000	170000	28000	
90	98000	16000+	140000	--	1400	31000	1300	--	220000	660000	200000	--
95	170000	16000+	250000	--	3400	16000+	2800	--	400000	--	350000	--
No. of samples	88	28 (1)	27	3	88	28	27	3	86	28 (3)	27	3

(1) 18 of 28 samples 16000+
(2) 14 of 79 samples TN
(3) 11 of 28 samples TN

TABLE IV

COLIFORM AND E C GAS POSITIVE MPN'S AND PLATE COUNTS AT PROBABILITY PERCENTILES 5, 10, 50, 90 and 95
Shucked Oysters as Received at Markets

Per-centile	Coliform MPN					E C Gas Positive MPN					Plate Count				
		New York City		New York State			New York City		New York State			New York City		New York State	
	Chicago	Southern	Northern	Southern	Northern	Chicago	Southern	Northern	Southern	Northern	Chicago	Southern	Northern	Southern	Northern
						Dec. - Jan. - Feb. - Mar.									
5	--	40	10	<18	<18	<18	<18	<18	<18	--	4800	880	--	--	--
10	--	100	29	<18	<18	<18	<18	<18	<18	--	11000	1600	--	--	--
50	--	3000	1300	320	130	<18	<18	<18	<18	--	180000	12000	4400	2300	460
90	--	90000	46000	12000	55000	190	74	125	86	--	2900000	92000	150000	60000	640000
95	--	240000	130000	34000	160000+	490	200	195	330	--	6400000	160000	400000	150000	5000000
No. of samples--		153	25	102	17	83	153	25	94	10	83	152	25	102	17
						Apr. - Sept. - Oct. - Nov.									
5	--	300	74	11	<18	<18	<18	<18	<18	<18	6800	2000	430	160	--
10	--	740	210	22	<18	<18	<18	<18	<18	<18	13000	3500	1000	380	710
50	--	19000	8000	1900	790	80	15	120	<18	<18	160000	26000	20000	8000	15000
90	--	160000+	290000	140000	83000	9800	2200	2400	560	2200	1800000	180000	400000	170000	320000
95	--	160000+	--	160000+	160000+	39000	8400	5700	1900	13000	3600000	330000	940000	340000	740000
No. of samples--		91	19	93	14	79	91	19	86	13	72	91	19	92	14

TABLE V

PERCENT ATTAINMENT OF COLIFORM MPN LIMITS
Shucked Oysters as Packed and as Received at Markets

Coliform MPN	As Packed			As Repacked		As Received at Markets				
							New York City		New York State	
	Virginia	Maryland	New Jersey	Maryland	New Jersey	Chicago	Southern	Northern	Southern	Northern
				Dec. - Jan. - Feb. - Mar.						
78	1	16	35	--	55	--	8	18	32	46
230	5	29	55	2.3	68	--	16	29	46	55
790	20	48	75	11	81	--	30	45	63	65
2400	44	66	88	31	89	--	38	60	76	73
16000	86	88	98	76	97	--	74	82	92	85
160000	99.5	98.4	--	98.6	--	--	93	96	98.6	--
No. of samples	114	165	36	29	24	--	153	25	102	17
				Apr. - Sept. - Oct. - Nov.						
78	--	3.5	--	--	--	--	1.5	5.2	18	27
230	1.4	10	--	0.2	--	--	4	11	27	37
790	9	26	--	1.9	--	--	10.4	21	40	50
2400	27	46	--	8.5	--	--	20	34	53	62
16000	76	81	--	45	--	--	48	60	73	79
160000	98.8	98	--	91.4	--	--	81	86	91	--
No. of samples	90	160	2	27	3	--	91	19	93	14

TABLE VI

PERCENT ATTAINMENT OF E C GAS POSITIVE MPN LIMITS
Shucked Oysters as Packed and as Received at Markets

EC + MPN	As Packed			As Repacked		As Received at Markets				
							New York City		New York State	
	Virginia	Maryland	New Jersey	Maryland	New Jersey	Chicago	Southern	Northern	Southern	Northern
				Dec. - Jan. - Feb. - Mar.						
78	91	89	79	76	93	82	90	85	90	--
230	95	94	92	80	95.6	91	96	96	94	--
790	98.2	97.5	98	84	97.4	96.6	98.5	99.5	97	--
2400	99.2	99	99.6	90	98.6	98.8	99.5	--	98.6	--
16000	--	--	--	99	--	99.9	--	--	99.7	--
160000	--	--	--	--	--	--	--	--	--	--
No. of samples	114	165	36	29	24	83	153	25	94	10
				Apr. - Sept. - Oct. - Nov.						
78	62	67	--	51	--	50	68	43	75	73
230	78	77	--	70	--	61	76	61	84	80
790	91	86	--	86	--	73	85	79	92	86
2400	96	92	--	94	--	82	91	90	96	90
16000	99.7	97.5	--	--	--	92	97	98.1	99	95
160000	--	99.5	--	--	--	98	99.3	--	--	--
No. of samples	90	160	2	27	3	79	91	19	86	13

TABLE VII

PERCENT ATTAINMENT OF PLATE COUNT LIMITS
Shucked Oysters as Packed and as Received at Markets

Plate Count	As Packed			As Repacked		As Received at Markets				
							New York City		New York State	
	Virginia	Maryland	New Jersey	Maryland	New Jersey	Chicago	Southern	Northern	Southern	Northern
				Dec. - Jan. - Feb. - Mar.						
5000	50	74	81	44	38	5	29	52	62	66
10000	65	83	93	57	53	9.5	46	62	72	70
50000	69	96	99.8	83	84	29	82	81	89	80
100000	95	98	99.98	93	91	40	91	87	93	83
500000	99.3	99.8	--	99	98.8	69	99.1	96	98.3	89
1000000	99.8	--	--	99.8	99.6	79	99.7	98	99.2	91
No. of Samples	112	163	36	29	23	83	152	25	102	17
				Apr. - Sept. - Oct. - Nov.						
5000	19	43	--	12	--	3.5	15	28	42	32
10000	32	57	--	24	--	7.5	27	38	54	43
50000	70	84	--	64	--	28	67	65	78	70
100000	82	91	--	79	--	41	81	75	86	79
500000	97	98.5	--	97	--	73	97.3	92	96	93
1000000	99	99.4	--	99	--	83	99.2	95	98	96
No. of Samples	89	153	2	27	3	72	91	19	92	14

PARTICIPATING AGENCIES

City of Chicago, Board of Health

Department of National Health and Welfare of Canada
  Food and Drug Directorate

Louisiana State Department of Health
  Division of Food and Drugs
  Division of Laboratories

Maryland State Department of Health
  Bureau of Environmental Hygiene
  Bureau of Laboratories

New Jersey State Department of Health
  Bureau of Food and Drugs
  Bureau of Laboratories

New York City Department of Health
  Bureau of Food and Drugs
  Bureau of Laboratories

New York State Conservation Department
  Bureau of Marine Fisheries
  Shellfish Management Unit

Virginia State Department of Health
  Division of Engineering

U. S. Department of Health, Education, and Welfare
  Public Health Service
    Bureau of State Services
      Division of Engineering Services
        Milk and Food Program
        Robert A. Taft Sanitary Engineering Center
          Milk and Food Research, Shellfish Sanitation Laboratory
          Statistical Services
      Regional Offices II and III
        Regional Engineers, Shellfish Consultants

APPENDIX NN

THE EFFECT OF COMMERCIAL HANDLING AND STORAGE ON THE BACTERIOLOGICAL QUALITY OF PACIFIC OYSTERS

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INTRODUCTION

The Shellfish Sanitation Laboratory conducted field and laboratory studies to determine the effect of commercial handling and storage on the bacteriological quality of Pacific oysters. These investigations were conducted primarily on the Pacific oyster Crassostrea gigas to obtain information similar to that obtained on the Eastern oyster. Information on the behavior of fecal organisms and plate counts in Pacific species was considered necessary for discussions that had been proposed for the 1961 Shellfish Sanitation Workshop on the applicability of these two indices to Eastern oysters and in anticipation of interest in the applicability of similar bacteriological criteria to the Northwest species.

This report covers three investigations. The first was a program of routine sampling and examination of shell and shucked Pacific oysters at commercial processing establishments representative of this area. This study has been in progress for more than two years, and testing has been done throughout a number of seasons. The second study was actually a bacteriological survey conducted by the Washington State Department of Health and involved routine sampling of commercial packs of oysters as received in the Seattle market area. The third investigation was a study of the bacterial changes that take place in commercial packs of shucked oysters stored at 3°C (37.5°F) and at -21°C (-6°F) under carefully controlled conditions.

Bacteriological tests conducted were coliform MPN, fecal coliform MPN, and standard 35°C plate counts after 48 hours incubation. In addition, the incidence of coliform types in the E C gas positive tubes from stored samples was determined by the E C IMViC completed test.

The laboratory procedures were those described in the proposed Recommended Procedures for the Examination of Sea Water and Shellfish. To insure that the methods and techniques used by all participants in the study were identical, the bacteriologist from the Washington State Department of Health spent two weeks at the Shellfish Sanitation Laboratory for indoctrination.

STUDIES IN PROCESSING PLANTS

A program to determine bacterial densities in oysters in commercial shucking establishments was conducted by the Shellfish Sanitation Laboratory. Shellstock samples were collected immediately before and after shucking and as packed in commercial containers after washing and sorting. Eighty-seven sets of samples were collected from December 1958 through September 1961 from 14 processing plants in the Purdy, Shelton-Olympia, and South Bend areas.

Results

The data were arrayed in ascending magnitude, and the 10, 40, and 90 percentile levels determined. This method was used because of an excessive number of indeterminately low E C MPN's. Since it was impossible to prepare probability plots of the E C test results, all data were treated similarly.

Coliform, EC MPN's, and 35°C plate counts at these percentiles are presented in Table I. The data were tested for seasonal variations in bacterial levels or changes during processing. Twenty-four sampling tours were made during the summer season, April through September. No overall differences were observed between the samples collected during the summer months and those collected during the colder months. Bacteriological results indicate a progressive but slight increase in coliform MPN and plate count during processing. Since there is a washing process between shucking and packing, the higher bacterial numbers in the packed samples could be significant.

Little if any change occurred in the fecal coliform MPN during processing. By comparison, the plate count and, to some extent, the coliform MPN did show increases. This substantiates the findings of the East Coast studies, which indicated that the fecal coliform MPN was a more stable index of acceptable handling for shipment and thus would serve as a more suitable index of the sanitary condition of the oysters as they were harvested.

Table II shows compliance with selected limits stated in the PHS manual for Eastern oysters at various stages of harvesting and processing and at the market. It is recognized that these limits are not applicable in all instances. For example, the manual requirement of a plate count of less than 100,000 applies to shucked Eastern oysters at the market and not to shell stock before shucking. Further, the manual requirement of an MPN of less than 78 applies to fecal coliform MPN's of shucked Eastern oysters at the market.

The data show a high degree of compliance with all criteria except the coliform MPN maximum of 230. Conformity of the shell stock with a coliform MPN of 230 was 39.3 percent, but all samples showed coliform MPN's less than 2400. It must be noted that coliform MPN of 230 applies to Eastern oysters as harvested in the New England area, not immediately before shucking.

A comparison between E C densities of packed Pacific oysters and the E C criteria for Eastern oysters at the market shows a degree of conformity. Of the samples 75.8 percent had an E C MPN of 78 or less, and 88.5 percent had an E C MPN of 230 or less. Only 11.5 percent of the samples had an E C MPN above 230.

The 35°C plate count groups of 5,000 and 10,000 show the degree of conformity with these arbitrarily selected densities and indicate the range of densities. Of the samples, 98.8 percent had plate count densities of 100,000 or less and 1.2 percent had plate count densities between 100,000 and 500,000.

MARKET STUDIES

Since November 1960, the Washington State Department of Health has conducted a survey of the bacteriological condition of shucked oysters at the wholesale market. To date 333 samples have been collected from various wholesale establishments and points of distribution in the Seattle area. As indicated, the bacteriological procedures were identical to those used by the Shellfish Sanitation Laboratory in the in-plant studies and in the studies on the effect of storage on the bacterial quality of shucked oysters.

Results

A preliminary frequency distribution of the data for market oysters on a year-around basis indicated variation. Sorting the data by months revealed that higher values occurred during the months of May, June, July, August, and September than during the months of December, January, February, March, and April. Therefore the data were subdivided into these two five-month seasons.

Bacterial densities at three percentile levels are presented in Table III. A difference between winter and summer samples can be seen. Coliform MPN's and 35°C plate counts are at higher levels during the summer season. Again no difference in E C densities was determined.

In order to compare the results of the plant studies with those of the market studies, coliform and E C MPN values and plate counts of the oysters as packed in commercial establishments were included in Table III. The plant samples are not related to the market samples in that both are not from identical lots; rather the plant samples represent a cross section obtained through the season from several shucking establishments. Likewise the market samples represent a cross section of shucked oysters throughout the season from representative distribution points.

In addition to increased levels during the summer period, the market samples showed significantly higher values than the plant samples during both seasons. Little if any change occurred in the fecal coliform MPN's, and the coliform MPN and plate count responded in a logical fashion. Although increases occurred during the winter, those occurring during the summer were greater.

STUDIES ON STORED OYSTERS

Eighteen lots of shucked Pacific oysters were collected from seven plants in three important shellfish growing areas in Washington. To investigate the possible influence of season, ten of the lots were collected in the winter and eight in the summer. Each lot consisted of sufficient oysters (approximately 2-1/2 gallons) to fill thirty-two 12-ounce cans. The oysters were washed, packed, and sealed by routine commercial methods. Sixteen of the cans were packed in crushed ice, returned to the laboratory, and stored in a dry refrigerator at 3°C. A standard refrigerator equipped with a thermoregulator and facilities for air circulation was used. Temperatures ranged from 1.5°C to 4°C with a median of 3.3°C. The remaining 16 cans of oysters, designated for frozen storage, were packed in ice and transported to blast-freezing facilities. The maximum time between collection and placement in the freezer was two hours. Frozen samples were transported to the laboratory in an insulated carrier and stored in a home-type freezer. Freezer temperature ranged from -20.6°C to -23.3°C with a median reading of -21°C.

Sampling schedules were as follows:

1. Refrigerated storage: 0 hour (1 to 2 hours after collection) 1, 2, 4, 7, 10, 15, and 20 days.

2. Frozen storage: 0 hour; 1 week, 1, 2, 3, and 6 months (This will be extended to include 9-month samplings).

Before the cans were opened, the can tops and the can opener were flamed with 95 percent alcohol. Approximately 5-ml aliquots of oyster liquor were withdrawn from cans refrigerated at 3°C for pH determinations. To avoid thawing prior to grinding, frozen samples were removed to a sterile aluminum pan and broken into pieces about an inch square with a sterile knife. Liquor from approximately 15 grams of sample thawed at room temperature was used for pH determinations.

In most instances, duplicate samples were collected . Sample dilution and grinding and bacterial testing were done in accordance with the methods described in the 1961 edition of the APHA Recommended Procedures for the Laboratory Examination of Shellfish in press. The pH determinations were made electrometrically on a Model G Beckman meter.

Results

Bacterial and pH changes in winter and summer lots of Pacific oysters are given in Table IV. Because of an excessive number of indeterminately low MPN's, average values could not be determined for the E C gas positive or E. coli type I organisms. To maintain uniformity, all bacterial results are expressed as maximum, median, and minimum values. Maximum and minimum MPNs show the ranges of variation that are largely attributable to lot differences. The pH values are arithmetic averages, in most instances, of a series of determinations from duplicate samples.

Changes in bacterial indices are illustrated in Figures 1 and 2, which present the median plate count, coliform, and E C MPN values listed in Table IV.

Initial E C MPNs were at approximately the same level in both winter and summer lots and showed similar patterns of change. During the first week of storage the densities of the E C gas positive organisms declined to near the lowest determinate MPN limit. The decline was numerically small, however, and represented a maximum of a two-tube difference. In the winter lots the E C MPN's remained unchanged from the seventh through the tenth day of storage and by the fifteenth day were indeterminately low. In the summer lots a slight peak occurred on the tenth day, but by the fifteenth day the MPN was again near the lowest determinate level and by the twentieth day was indeterminately low. Because of a close similarity between E C and E. coli type I results the latter MPNs were not plotted.

Although changes in the coliform content of the winter and summer lots were quite similar through the first 4 days of storage the overall pattern was very different; there was some fluctuation in the first 4 days but no significant numerical changes. In the summer lots this persisted through the first 7 days of storage. Throughout the remaining 13 days of storage the median coliform content increased progressively to a level approximately 1-1/2 log cycles above the initial median value. In contrast, the coliform content of winter lots increased about four-fold from the fourth to the seventh day and remained at this level through the tenth day. During the latter half of storage, the median values declined progressively until the MPN on the twentieth day was approximately one-third that of the zero-hour value.

Although the 35°C plate counts in both the winter and summer lots increased significantly, the patterns of change during the first week of storage were dissimilar. Following a lag period of 2 days the plate counts in the winter lots increased rapidly throughout the remainder of storage. In contrast, the lag phase of the summer lots extended over the first 7 days. Throughout the remaining 13 days, increases were substantial and paralleled those in the winter lots.

Table IV shows a gradual decrease in the average pH in both winter and summer lots, from an initial value of approximately 6.5 to a final pH of about 5.7.

Table V presents the average pH, the maximum, median and minimum E C, E. coli type I, and coliform MPNs, and the standard plate counts for 8 lots of oysters collected in the winter and stored at -21°C; samples were taken at various intervals of storage up to 6 months. To illustrate changes in the bacterial indices, median coliform and E C MPNs, and plate counts from Table V are illustrated in Figure 3. Although the patterns in Figure 3 are different, all three indices declined in density.

The decline in E C density occurred in the first week of storage and was relatively small, decreasing from an MPN of 59 to 20. Throughout the remainder of the 6-month storage period the E C content was practically unchanged. This pattern is similar to that for the oysters stored at 3°C. The E. coli type I content of the oysters was initially low (MPN 20) and remained at this level for 1 month. Beyond 1 month the E. coli MPNs were indeterminately low. A small decrease in coliform content occurred slowly and progressively throughout storage.

Following a reduction of 50 percent in the first week, the plate count increased during the next 3 weeks to a level approximating the median zero-hour count. For the next 2 months the counts remained relatively unchanged and then dropped sharply between the third and sixth months. Over the 6-month period the median plate counts decreased to 23 percent of the zero-hour value. The pH of the samples remained practically unchanged, varying by only 0.1 of a pH unit throughout storage (Table V).

SUMMARY

Bacterial changes in commercially handled oysters were investigated in a cooperative study by the Shellfish Sanitation Laboratory and the Washington State Department of Health. Routine sampling programs were established in commercial shucking establishments and in the Seattle wholesale market area. The shucking plants were under surveillance by the State Health Department and represent average acceptable sanitary conditions in this type of establishment. Samples from the market area were catch samples at representative points of distribution in the Seattle area.

Progressive but slight increases in coliform MPNs and plate counts were noted during the shucking and packing operation. In contrast, little if any changes in fecal coliform MPNs were noted. These behaviors are as would be expected in shucking houses where operation is satisfactory.

In general, the levels of coliform MPNs and plate counts were somewhat higher in oysters at the market than as packed in the shucking plants. The fecal coliform MPNs showed little if any change. Thus, the fecal coliform organisms appear to give better representation, at the market, of the bacterial condition of the oysters as they were harvested or packed. The fact that a few fecal coliform MPNs were high in market samples suggests that the fecal coliforms might be useful in detecting seriously improper treatment of the product during handling or shipment.

Changes in these indices during storage at 3°C and -21°C were also studied. Results indicate that during storage at 3°C the bacterial index that showed a more consistent pattern of change and was influenced least by duration or conditions of storage or by season was that segment of the coliform group enumerated by the E C test. The present data are in close agreement with results obtained in the field and support the view that the E C gas positive organisms are the best index to indicate fecal contamination in shucked Pacific oysters. Results from the plant and market studies and the storage studies indicate that a moderate increase in plate count can be expected even under acceptable conditions of processing, handling, and refrigeration. Indications are, however, that sudden increases in plate count are indicative of improper handling or refrigeration at the processing or marketing levels and of the beginning of spoilage.

At a storage temperature of -21°C, slight, comparable decreases in the coliform and E C content were noted. Use of the E C test to evaluate frozen samples is suggested, however, since it is of value in assessing the sanitary quality of oysters as harvested and at the market.

The slight changes in the bacterial indices that occur within 6 months of storage indicate the necessity for extending the study an additional 3 to 6 months to determine more conclusively the fate of the index organisms under frozen conditions. Sufficient samples are on hand to extend the program to include examination after 9 months to 1 year of storage.

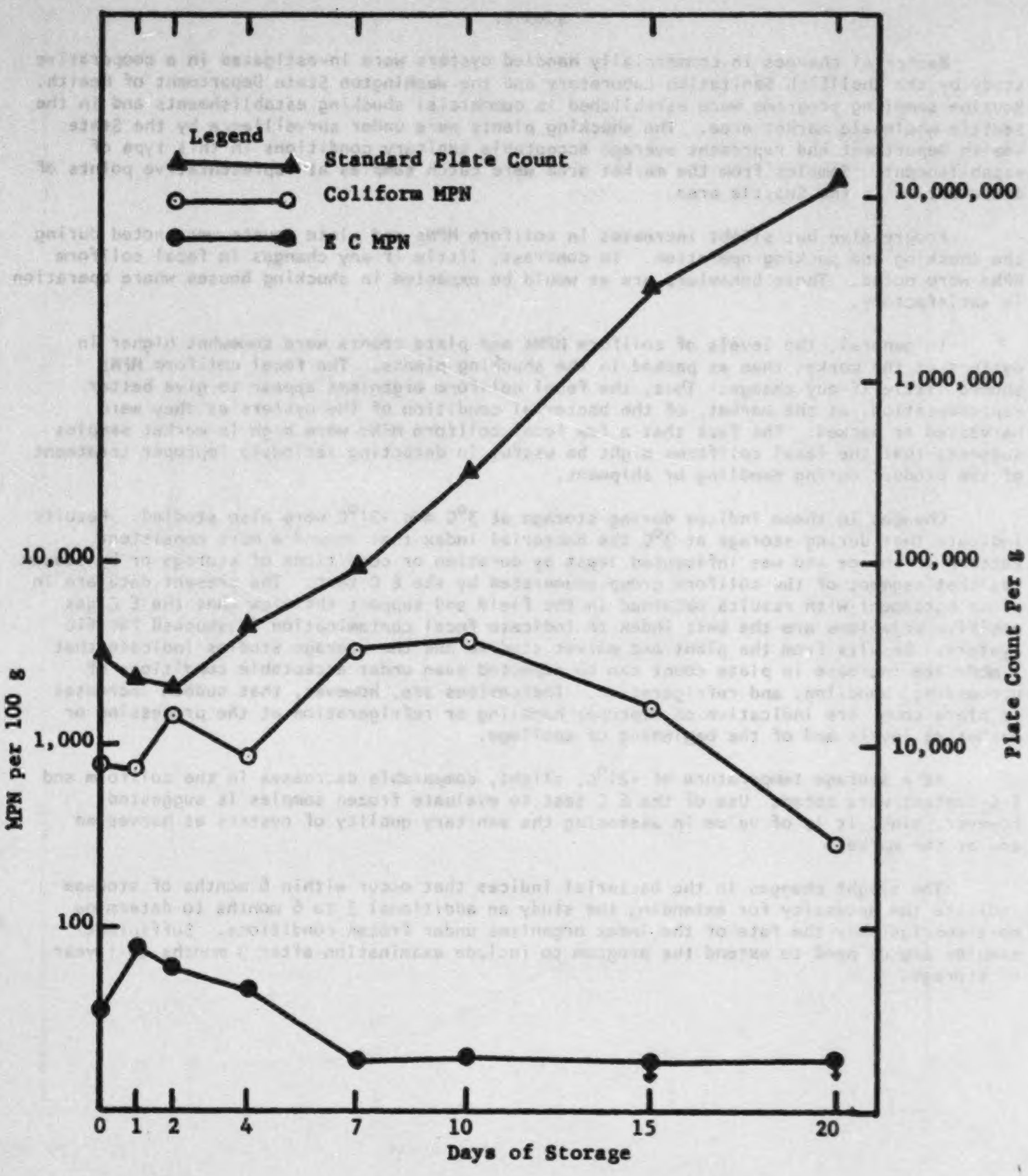

FIG. 1. CHANGES IN BACTERIAL INDICES IN WINTER LOTS OF PACIFIC OYSTERS STORED AT 3°C

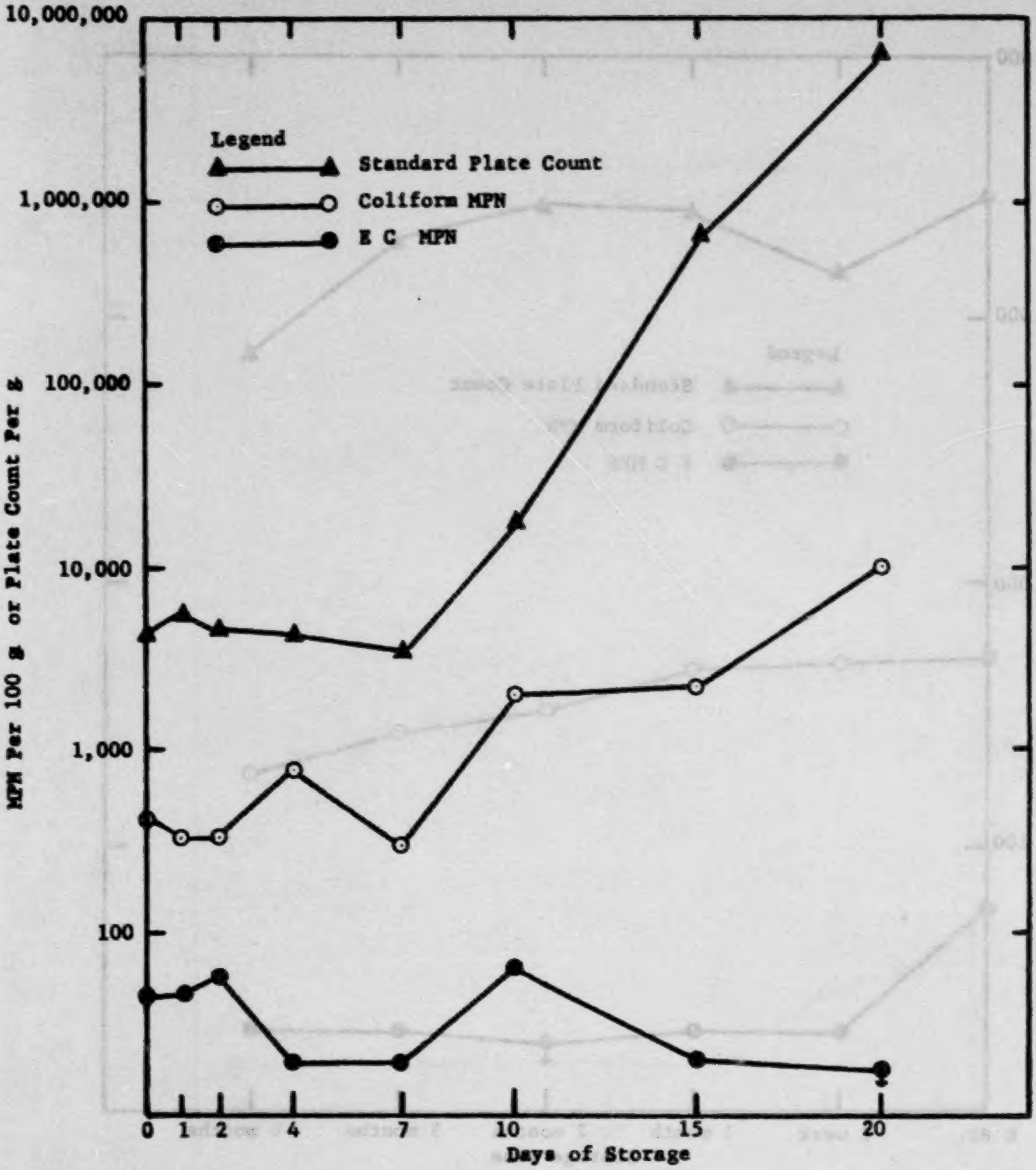

FIG. 2. CHANGES IN BACTERIAL INDICES IN SUMMER LOTS OF PACIFIC OYSTERS STORED AT 3°C

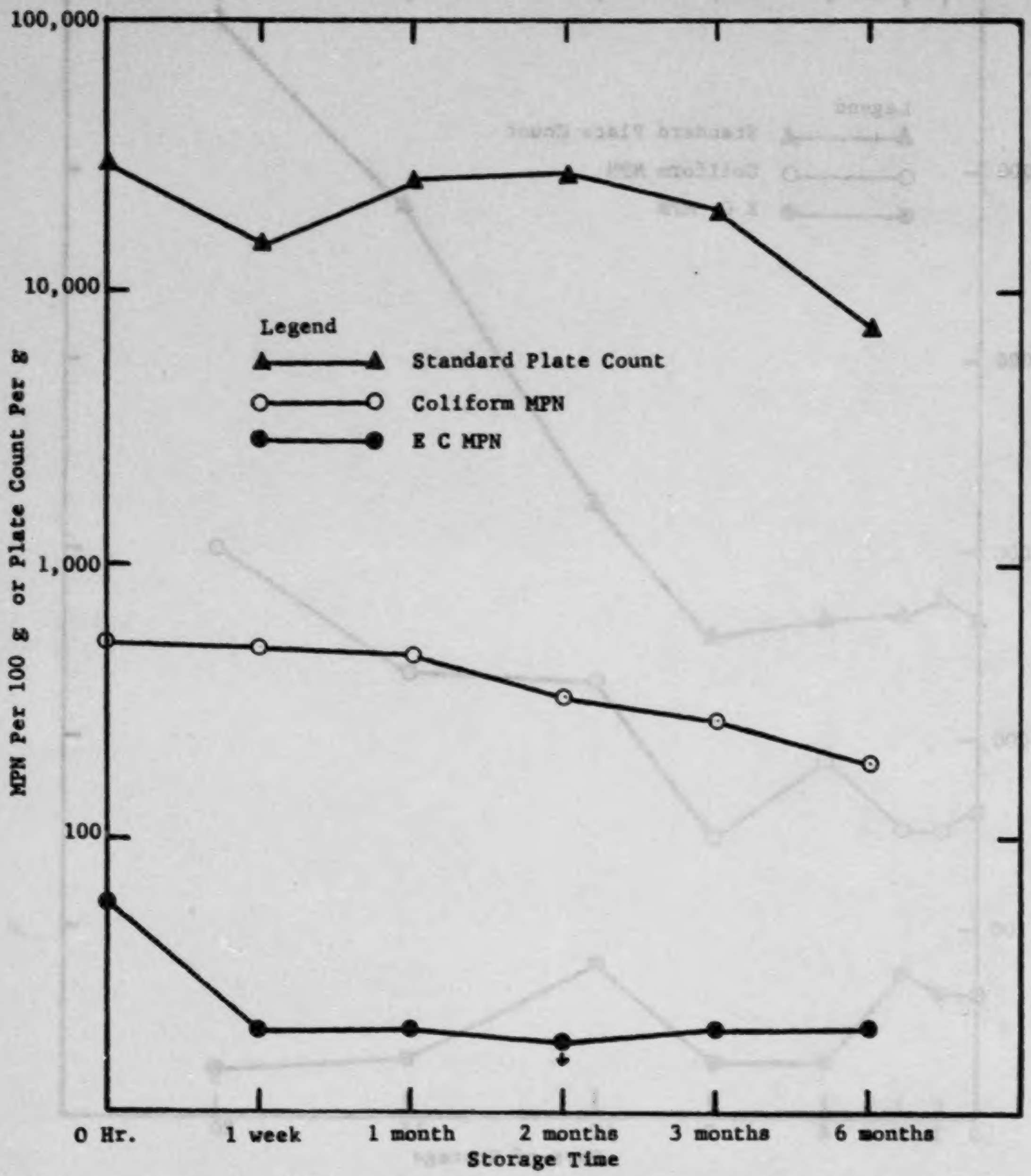

FIG. 3. CHANGES IN BACTERIAL INDICES IN PACIFIC OYSTERS STORED AT MINUS 21°C

TABLE I. BACTERIAL DENSITIES OF COMMERCIALLY PROCESSED PACIFIC OYSTERS

	Coliform MPN per 100g			E C MPN per 100g			35°C Plate count per g		
	Shell	Shucked	Packed	Shell	Shucked	Packed	Shell	Shucked	Packed
Maximum	2,400	5,400	11,000	490	790	1,700	42,000	80,000	180,000
Minimum	20	30	40	<18	<18	<18	180	60	300
10 percentile	51	110	110	22	<18	<18	370	360	560
50 percentile	330	330	460	45	20	40	1,100	2,400	5,900
90 percentile	1,300	1,700	3,300	230	220	330	9,000	14,000	32,000
Number of samples	87	68	87	87	68	87	87	68	87

TABLE II. ATTAINMENT OF MPN AND PLATE COUNT LIMITS[a/] IN COMMERCIALLY PROCESSED PACIFIC OYSTERS

	% Attainment							% Attainment		
	Coliform MPN			E C MPN				35°C Plate count		
MPN per 100g	Shell	Shucked	Packed	Shell	Shucked	Packed	Plate count per g	Shell	Shucked	Packed
78	4.6	10.3	4.6	67.3	73.5	75.8	5,000	82.7	70.6	47.1
230	39.3	34.3	36.7	90.8	92.6	88.5	10,000	94.5	83.9	73.7
2,400	100	95.2	87.3	100	100	100	50,000	100	98.5	93.1
16,000	-	100	100	-	-	-	100,000	-	100	98.8
							500,000	-	-	100
Number of samples	87	68	87	87	68	87		87	68	87

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a/ For Eastern oysters (from PHS manual).

TABLE III. BACTERIAL DENSITIES OF PLANT AND MARKET PACIFIC OYSTERS

Percentile	Coliform MPN per 100g			E C MPN per 100g			35°C Plate count per g		
	Plant	Market		Plant	Market		Plant	Market	
		Winter a/	Summer b/		Winter a/	Summer b/		Winter a/	Summer b/
10	110	490	490	<18	<18	<18	560	500	1,000
50	460	3,500	9,200	40	40	20	5,900	5,000	10,000
90	3,300	92,000	920,000	330	230	230	32,000	100,000	500,000

a/ Winter 11-8-60 to 5-1-61

b/ Summer 5-9-61 to 10-9-61

TABLE V. CHANGES IN THE BACTERIAL INDICES AND pH IN PACIFIC OYSTERS STORED AT MINUS 21°C

Storage time	MPN per 100g									35°C Plate count per g			pH
	Coliform			E C gas positive			*E. coli* type I						
	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	
0 Hour	7,900	510	110	790	59	<18	790	20	<18	100,000	30,000	4,200	6.5
1 Week	7,000	500	20	78	20	<18	78	<18	<18	79,000	15,000	5,300	6.5
1 Month	3,300	470	45	230	20	<18	230	20	<18	480,000	25,000	2,300	6.5
2 Months	1,700	330	20	45	<18	<18	45	<18	<18	560,000	27,000	9,000	6.5
3 Months	2,400	270	20	110	20	<18	78	<18	<18	250,000	20,000	7,200	6.6
6 Months	3,400	190	20	61	20	<18	20	<18	<18	260,000	7,400	2,700	6.6

TABLE IV. CHANGES IN BACTERIAL INDICES AND pH IN PACIFIC OYSTERS STORED AT 3°C

Days of storage	MPN per 100g Coliform Maximum	Coliform Median	Coliform Minimum	E C gas positive Maximum	E C gas positive Median	E C gas positive Minimum	E. Coli type I Maximum	E. Coli type I Median	E. Coli type I Minimum	35°C Plate count per g Maximum	Median	Minimum	pH
							Winter Lots						
0	7,900	790	45	790	35	<18	790	19	<18	100,000	31,000	4,400	6.5
1	9,500	740	130	790	78	<18				200,000	23,000	2,900	6.2
2	17,000	1,400	170	1,300	60	<18	230	20	<18	390,000	21,000	3,800	6.1
4	54,000	850	40	490	45	<18	140	20	<18	340,000	45,000	5,200	6.0
7	160,000	3,300	78	310	18	<18	310	18	<18	1,200,000	99,000	15,000	5.9
10	1,600,000	3,800	61	220	20	<18	110	20	<18	12,000,000	310,000	14,000	5.7
15	92,000	1,600	82	170	<18	<18	170	<18	<18	81,000,000	2,400,000	21,000	5.5
20	350,000	290	37	45	<18	<18	45	<18	<18	460,000,000	13,000,000	14,000	5.7
							Summer Lots						
0	2,300	410	170	790	45	<18	790	43	<18	19,000	4,300	400	6.6
1	5,400	330	93	170	45	<18	130	20	<18	41,000	5,800	420	6.4
2	4,900	330	130	170	59	20	170	45	<18	39,000	4,600	370	6.2
4	4,900	790	20	790	19	<18	490	18	<18	41,000	4,200	900	6.1
7	4,900	300	45	490	20	<18	170	<18	<18	64,000	3,500	600	5.8
10	12,000	2,000	210	950	64	<18	78	20	<18	180,000	18,000	1,300	5.6
15	160,000	2,200	45	170	20	<18	110	20	<18	4,700,000	66,000	3,400	5.5
20	>160,000	10,000	130	130	<18	<18	130	<18	<18	67,000,000	670,000	40,000	5.6

APPENDIX O

THE CHROMATE COLOR TEST

By

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The chromate color test is a freshness test for oysters. It can be used to estimate storage time at 32° to 42° F, i.e., the time for shucking to the time when the product is tested, or if the storage time is known, it can be used to detect whether the storage temperature has exceeded 32° to 42° F. Evidence has been developed to show the usefulness of the test as an objective criterion (1) for assuring the quality of the packer's product as it leaves the plant and (2) for evaluating the time-temperature history of the product after shipment. The test also should be helpful to repackers in estimating how long oysters were held by the original shucking plant. The test is simple and can be performed with a little practice by a person untrained in chemical work.

The development of the test and the laboratory evaluation have been reported (1). The test is run on a 2-ml aliquot of a 1:16 dilution of the liquor surrounding the oysters, and the results are expressed in mg Chromium III produced. This corresponds to the concentration of reducing substances (RS) in the oyster liquor. Under test conditions, the liquor taken from oysters within 15 minutes of the time they are packaged will produce less than 1 mg Chromium III. With storage at 32° to 42° F, the liquor taken from oysters that have been packaged 1 to 2 days will produce 2 mg Chromium III or less; those that have been packaged 3 to 4 days will produce 3 mg Chromium III or less; and those that have been packaged 5 to 6 days will produce 4 mg Chromium III or less. If the shucked oysters are exposed to higher temperatures, they will have higher concentrations of RS at any given time after shucking.

Commercially processed Gulf Coast oysters were used in the development of the test; Chesapeake Bay oysters were used in the laboratory evaluation, which consisted of two experiments. In one, the temperature was controlled (32° to 42° F) and the test was run at known time intervals after processing. For the other, aliquots of oysters that had been shucked 24 to 36 hours were subjected to four different temperatures: 32°, 59°, 77°, and 95° F. The results of these experiments indicate that during the first 6 days the increase in RS in oysters held at 32° F is sufficiently rapid that the test can be used to determine the number of days the oysters have been shucked and that there is a direct relationship between storage temperature and the rate of production of RS. The R.S. concentration doubled in the aliquot held 4 hours at 95° F but showed no significant increase in the aliquot held for the same length of time at 32° F.

A cooperative field study was carried out during the 1959-60 oyster season to determine the expected rate of increase of RS in shucked oysters during commercial shipment to the retail market (2). The results of this study verified the usefulness of this test in evaluating the time-temperature history of shucked oysters as received after shipment.

During the 1960-61 oyster season additional tests were carried out on the effect of certain processing variables on the subsequent rate of increase of RS in shucked oysters stored under optimum temperature conditions. Some of the variables studied included (1) "blowing" process vs. light wash, (2) temperature of water used to wash oysters, (3) size of oysters, (4) temperatures at which shell stock were held during the time interval between harvesting and processing, (5) salt concentration of water used in processing oysters, and (6) effect of prolonged exposure to atmospheric oxygen.

The first three of these variables had no effect on the subsequent rate of increase of RS in shucked oysters held under identical temperature conditions. When oysters were soaked in water containing 1% NaCl or when salt was added to the finished pack (7.5 g NaCl to 2 lb oysters), there was a slight inhibition in the production of RS during subsequent storage; however, in all cases the Chromium III values were within 1 mg of those observed for samples processed in the conventional manner.

Oysters stored in containers with considerable air space showed a significantly greater rate of increase of RS than aliquots stored in sealed containers with almost no air space. One quart of shucked oysters stored in a gallon container assayed 4.5 mg Chromium III after 4 days of storage, whereas an aliquot from a sealed pint container assayed at 2.5 mg Chromium III after identical time and temperature storage.

The temperature at which the shell stock was stored before it was shucked had a decided effect on the rate of increase of RS in the shucked product. Shell stock held near freezing temperature, so that the oysters were at 34° F immediately after shucking, assayed 3.5 mg Chromium III after 2 days of storage; in contrast, shell stock held at higher temperatures, so that the oysters immediately after shucking were at 60° to 65° F, assayed 1 mg Chromium III after 2 days of storage. How holding shell stock at higher storage temperatures affects the rate of increase of RS in the shucked product has not been investigated; however, this could be the cause of the large variation observed in samples of oysters harvested from the Gulf Coast, since in this area it is occasionally very difficult to prevent short-time exposure of shell stock to high temperatures.

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APPENDIX OO

REPORT ON THE COOPERATIVE FIELD STUDY ON THE CHROMATE COLOR TEST FOR DETERMINATION OF THE TIME-TEMPERATURE HISTORY OF RAW SHUCKED OYSTERS

By

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Introduction

The chromate color test (1) was developed to measure the concentration of reducing substances (RS) in the liquor surrounding shucked oysters. Laboratory evaluation of the test has shown that the rate of increase of RS in commercially processed oysters is dependent on storage temperature. With efficient cooling and careful control of the storage temperature at 0°-5°C, RS increases gradually at a predictable rate, making it possible to estimate the age of the shucked product from the results of the chromate color test. At higher temperatures the rate of RS accumulation is increased. The predictable rate of RS increase at 0°-5°C makes it possible to determine whether oysters of known age have been held at temperatures higher than 0°-5°C. This cooperative field study was set up to determine the expected rate of increase of RS in shucked oysters during commercial shipment to the retail market.

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Since raw oysters are consumed in all areas of the United States, oyster meat is frequently removed from the shell and packaged in sealed pint, 12-ounce or gallon containers for ease in shipment to interior areas. Antibiotics cannot be used to protect this product, since it is frequently consumed without cooking. Raw shucked oysters are so perishable that they become organoleptically unacceptable if held a few hours at temperatures higher than 15°C; however, if processed under optimum sanitary conditions, cooled efficiently, and held at 0°-5°C, they have a shelf life of a month or longer.

The commercial shucking procedure involves considerable handling of the raw product. Oysters are harvested either by tonging or dredging and may be held out of the water several days before they are shucked. Most shucking houses provide cover during this storage so that the oysters will not freeze during the winter months; however, they seldom have facilities for keeping the shell stock cool during warm weather. Customarily the oysters are shucked by hand into gallon buckets containing about one quart of water. The time required to shuck sufficient shell stock to yield one gallon of oyster meat is, of course, dependent on the size of the oysters and the skill of the shucker. A highly skilled worker can shuck one gallon of oysters in about one hour. For comfort the shucker must work at a room temperature of about 20°C.

In the Gulf Coast area, shucked oysters are generally drained on a strainer, washed with a spray of tap water, packaged, and immediately cooled with crushed ice. Along the East Coast it is customary to dump the gallon lots of shucked oysters into a vat, of perhaps 100-gallon capacity, that has been partially filled with tap water. When this vat is approximately one-fourth filled with shucked oysters, a stream of compressed air is passed up through the vat for approximately 30 minutes. Water is allowed to flow into the vat simultaneously so that the solid material, or scum, that accumulates on the surface overflows the side of the container. After this the oysters are lifted out onto a strainer, drained, packaged, and cooled.

Long-distance shipment is frequently involved before packaged oysters reach the retail market. In some areas the shucked product is transported to a second processing plant in 5-gallon containers and there repackaged into smaller containers for the retail market. The efficiency in handling this very perishable product, from shucking to retailing, is frequently assessed solely on the basis of an organoleptic examination. This cooperative study is concerned with an evaluation of the chromate color test for the assessment of the quality of this product. In the field adaptation, the chromate color test was run on diluted liquor samples; the resultant concentration of RS was determined by comparison of the color of the test solution with visual standards.

Equipment

A field kit was designed to carry all of the required glassware and equipment for the chromate color test. The kit is a wooden box 19-1/4 by 10-1/2 by 10-1/4 inches and weights 24 pounds. The lid and one side open up to make a portable laboratory (see Figure 2). The kit is stocked with the following equipment and glassware:

1. Sulfuric acid dispenser calibrated to deliver exactly one milliliter of concentrated sulfuric acid.
2. Polyethylene bottle (16 oz.) for distilled water.
3. Polyethylene bottle (2 oz.) for the standard glucose solution.
4. Polyethylene bottle (2 oz.) for the dichromate solution.
5. Fifteen test tubes (150 mm by 25 mm) with black plastic caps for preparation of diluted liquor samples.
6. Twenty-one test tubes (150 mm by 16 mm) for the color test.
7. One set of visual standards.
8. The following pipets:
   a. Fifteen pipets (1 ml) for removing liquor from oysters and for measuring the dichromate solution.
   b. Two volumetric pipets (15 ml) for measuring the 15-ml aliquots of distilled water.
   c. Fifteen pipets (2 ml) for measuring the aliquots of diluted liquor for use in the chromate color test.
9. Thermometer
10. Minute timer

Procedure

Mix the oyster sample that is to be tested. For the sealed pint and gallon containers, this may be done simply by inverting the sample several times. If a partially filled container is being used, however, the contents should be stirred gently.

Transfer 1 ml of the liquor to a test tube (150 mm by 25 mm) by means of a pipet. The viscosity of the oyster liquor makes it essential that the outside of the pipet be wiped off before the sample is delivered. Duplicate dilutions should be prepared.

Add 15 ml of distilled water to the aliquot of liquor and mix thoroughly.

Transfer a 2-ml aliquot of the diluted liquor to a test tube (150 mm by 16 mm).

Add 1 ml of the dichromate solution, and slightly agitate the contents by tapping the tube with the fingers.

Add 1 ml of concentrated sulfuric acid from the dispenser; allow sufficient time for the viscous acid to flow from the dispenser. Rotate the tube while adding the acid so that mixing is instantaneous. Mix contents thoroughly by shaking.

Set the timer for 10 minutes (using the calibrated gold mark on the timer) immediately after adding the acid.

Ten minutes after adding the acid, compare the color of the solution with the visual standards.

Since the depth of the solution is greater than the diameter of the tube, matching the colors is facilitated by looking down at the solutions at an angle (see Figure 1). Matching the sample and the particular standard is easier if the visual standard with slightly less and the one with slightly more blue color are held on either side of the sample. Greater precision is attained usually if a white background and indirect light are used.

Initial testing was done at three Chesapeake Bay area shucking houses, A, C and D, as the samples were "tagged" for inclusion in commercial shipments to five different retail outlets. The "tagging" of the samples consisted of placing pint, half-pint, 12-ounce samples in polyethylene bags and designating the commercial shipment to be used. The "tagged" samples were then picked up at the retail markets, packed in crushed ice, and transported to the receiving laboratories for testing. The retail outlets were located in Syracuse and New York City, New York, Indianapolis, Indiana, and Cincinnati, Ohio.

In addition, commercial shipments of oysters from two Chesapeake Bay area shucking houses, A and B, were sampled on arrival at four retail outlets. At the retail outlets a random composite sample was prepared by combining either 8 1/2-pint containers, six 12-oz. containers or four pint containers and was shipped to a receiving laboratory in Cincinnati for testing. The retail outlets were located in Dayton, Ohio; Detroit and Grand Rapids, Michigan; Carbondale, Illinois; and Wisconsin.

Results

Between October 19, 1959, and May 5, 1960, a total of 57 samples of standard and select oysters were tested at the shucking houses, A, B, C, and D. Forty-nine of these samples were "tagged" and included in commercial shipments to retail outlets. In most cases the oysters were tested immediately after processing and packaging in half-pint, pint, and 12-ounce containers; however, for shipments from shucking house C, the oysters were packaged initially in five-gallon cans and later repackaged in the small-sized containers for fulfillment of the retail order. These samples were "tagged" and tested at the time the small containers were being packaged.

The results for the 57 samples are summarized in Table 1. The range of initial readings for these samples was 0 to 1.5 mg Cr III. Sixty-eight percent gave values of 0.5 mg Cr III or less. Of the 14 percent that gave values of 1.5 mg Cr III, the majority (five out of a total of eight samples) were from the shucking house that initially packed the oysters in five-gallon containers. The tests were made while the oysters were being repacked for the retail market.

The data from the receiving laboratories are summarized in Table 2. Twelve "tagged" samples of standard oysters and four "tagged" samples of selects were tested one to two days after packaging; these samples contained sufficient reducing substances (RS) to produce 1 to 1.5 mg Cr III in the chromate test. Twelve samples of standards, both "tagged" and composite, when tested three to four days after packaging gave readings ranging from 1 to 3 mg Cr III. Five "tagged" samples of standards were tested five to six days after packaging; these contained sufficient RS to produce 3 to 4 mg Cr III. Six composite samples of standards of five to six days after packaging gave readings between 2.5 and 4.5 mg Cr III.

On the basis of these data and the results from the laboratory evaluation of the rate of increase of RS in oysters held at 0 to 5°C (1), the maximum values to be expected in shucked oysters that are cooled efficiently and held at 0 to 5°C are given in Table 3. No attempt is made to estimate the age of shucked oysters held longer than five to six days after packaging. In all samples of shucked oysters the rate of increase of RS decreases with time if the temperature is held constant. In other words, the concentration immediately after packaging (less than 1 mg Cr III) doubles during the first two days of storage at

0 to 5°C. It increases by about fifty percent during the third to the fourth day of storage and by about thirty-three percent during the fifth to sixth day of storage, and then tends to remain at about the same level during subsequent storage.

Discussion

In the course of this cooperative study some data were also accumulated on variables, other than storage temperature, that affect the rate of increase of RS in the liquor of shucked oysters. The laboratory evaluation (1) indicated that no relationship exists between the results of the chromate color test and the shucking house, the size of the container in which the oysters are packaged, or the area from which the oysters are harvested. The scattered bacteriological data accumulated during this field study verify these previously reported observations concerning the lack of correlation between the initial population of viable microorganism and the results of the chromate color test.

In all cases when composite samples of standard and select oysters were held under similar storage and time conditions, the selects gave higher readings in the chromate test. In order to gain more information on this apparent effect of oyster size on the concentration of RS, samples of standard and select oysters were tested at shucking house A and held there under identical storage conditions. Three days after packaging, the standard sample produced 1.5 mg Cr III and the select sample 2.5 mg. When the test was repeated on a later date, the select oysters gave an initial reading of 0.5 mg Cr III and the standard oysters, 0.5 - 1 mg Cr III. These samples were "tagged" and included in a shipment to a retail outlet. After seven days the standard sample read 2.5 mg Cr III, and the select sample, 2 mg Cr III. Two weeks later, this experiment was repeated; freshly packaged standard and select oysters both gave readings of 0.5 mg Cr III. After two days, both samples gave readings of 1.5 mg Cr III. The two samples were held in crushed ice for an additional five days, and at this time both gave readings of 2.5 mg Cr III. These results indicate that oyster size has little or no effect on the rate of increase of RS during storage. The higher readings for the select oysters in the composite samples (see Table 2) must have been caused by the slower less-efficient processing frequently used when the larger-sized oysters are in a great minority.

In a study of the effect of "washing" on the concentration of RS, two 1-pint samples of oysters were held at 0°-5°C at shucking house A for ten days. One sample was washed thoroughly on a skimmer. At the time the washed sample was repackaged, it produced 1 mg Cr III; at the same time the unwashed control sample produced 2.5 mg Cr III. Both samples were put into a commercial shipment to a retail outlet. Two days later the control sample read 3 mg Cr III and the washed sample read 2.5 mg Cr III. These data indicated that washing only temporarily reduces RS concentration.

Some indication of the time interval between the shucking, blowing, and packaging of oysters and the initial testing was ascertained from the temperature of the oysters at the time they were tested. It was assumed that optimum conditions of storage temperature were employed and that any sample above 5°C had not been stored long enough to cool to 0° - 5°C. In Table 4 the results of tests of 57 samples at the four shucking houses have been grouped according to temperature. Of the 20 samples tested while the oysters were at temperatures reading higher than 10°C, i.e., immediately after packaging but before cooling, seventeen gave readings of 0.5 mg Cr III in the chromate color test. In contrast eleven of the fourteen samples that had been cooled to temperatures lower than 5.5°C gave readings of 1 to 1.5 mg Cr III. These results indicated that most samples of shucked oysters will produce only 0.5 mg Cr III or less if tested immediately after packaging.

Two samples of oysters with unknown time-temperature history were observed during the course of this study. A reading of 9.5 mg Cr III was reported on a spoiled sample of 12-day old oysters that had been lost in transit, and one of the retail outlets used in these tests received a sample of "tagged" oysters that gave a reading of 6.5 mg Cr III after only two recorded days of storage. Oyster samples that were arbitrarily and knowingly maltreated during these tests gave readings of less than 5 mg Cr III, however, even after they were obviously spoiled. These data indicate that as yet unknown variables cause very high readings in "old" shucked oysters.

Summary

Raw shucked oysters were assayed for their concentration of reducing substances (RS), as measured by the chromate color test under conditions of known time lapse after packaging and under the storage temperature used by commercial handlers.

Of the 57 samples tested at four shucking houses during packaging for shipment to retail outlets, 36 percent gave readings of 0.5 mg Cr III or less. All the samples gave readings of 1.5 mg Cr III or less. The majority of the oysters that gave the higher readings were from one shucking house that initially packaged the oysters in large containers and later repackaged them in smaller containers for retailing.

After the initial chromate color test, the samples were "tagged" and included in commercial shipments to five retail outlets. At six additional retail outlets composite samples were collected from the commercial shipments of one of the participating shucking houses, and these samples were again assayed.

The 16 samples of standard and select oysters that were tested one to two days after packaging contained sufficient RS to produce 1 to 1.5 mg Cr III. The 12 "tagged" and composite samples of standards that were tested three to four days after packaging gave readings of 3 to 4 mg Cr III and six composite samples, 2.5 to 4.5 mg Cr III.

The composite samples usually consisted of both select and standard oysters of the same age. In all cases the select oysters contained a higher concentration of RS than the standards. Additional studies indicated, however, that the size of the oysters had no significant effect on the rate of increase of RS during storage. Thus, variables during processing of the larger-sized oysters must have caused the higher RS concentrations in the composite samples.

Acknowledgements

The author is deeply indebted to Mr. John Hope, Bureau of Shellfish Sanitation, Commonwealth of Virginia, Department of Health, and Mr. L. F. Hobbs, State of Maryland Department of Health for the initial testing at the shucking house and the "tagging" of the samples for subsequent inclusion in commercial shipments to the designated retail outlets.

She wishes to acknowledge the very valuable assistance of the following persons who ran the tests at the receiving laboratories: Mrs. Joan Keene and Mr. Jack Foehrenbach of the State of New York Conservation Department, Mr. Glen E. Weber and Dr. Sara L. Bell of the Indiana State Board of Health, Mr. Cleo Hamilton of the R. A. Taft Sanitary Engineering Center, and Miss Emilie Stegemeyer of the Laboratories Division of the Kroger Food Foundation.

The cooperation of the following shucking houses is appreciated for making available shucked oyster samples of known time and temperature history for use in these studies: Ballard Fish and Oyster Co., Norfolk, Virginia; H. B. Kennerly and Son, Inc., Nanticoke, Maryland; J. H. Miles and Co., Inc., Norfolk, Virginia; and the Todd Co., Cambridge, Maryland.

The cooperation of the following retail markets is appreciated for permitting the inclusion of "tagged" samples in their shipments of shucked oysters: Atlantic and Pacific Tea Co., New York City; Central Oyster House, Cincinnati, Ohio; Colonial Stores Cincinnati, Ohio; J. M. Raichelson, Syracuse, New York; and Columbia Fish and Oyster Co., Indianapolis, Indiana.

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Table 1

Reducing Substances in Oysters Immediately After Packaging, as Indicated by Chromate Color Test

	Number of Assays at Shucking House				
mg Cr III	A	B	C*	D	Total Number of Assays
0 - 0.5	18	3	14	4	39
1	3		4	3	10
1.5	2		5	1	8
					57

*Many of the samples from this shucking house were stored initially in five-gallon containers. Tests were run on the oysters as they were repackaged in half-pint, pint, and 12-ounce containers.

Table 2

Reducing Substances in Oysters After Shipment to Retail Outlets, As Indicated by Chromate Color Tests

	Time Lapse Between Packaging and Testing							
	1-2 days		3-4 days		5-6 days		7-12 days	
	Tagged	Composite	Tagged	Composite	Tagged	Composite	Tagged	Composite
Standards								
No. of Samples	12	-	8	4	5	6	8	8
Range (mg Cr III)	1-1.5	-	1-3	2.5-3	3-4	2.5-4.5	1.5-4	3-5.5
Selects								
No. of Samples	4	-	-	5	3	6	4	6
Range (mg Cr III)	1-1.5	-	-	3.5-4.5	1.5-3	3.5-4.5	2-4.5	3.5-6

Table 3

Maximum Reducing Substances in Stored Oysters*, as Indicated by Chromate Color Test

Days after shucking	RS, mg Cr III
< 1	≤ 1
1-2	≤ 2
3-4	≤ 3
5-6	≤ 4

* Shucked oysters cooled efficiently and held at 0° - 5°C.

Table 4

Correlation of Temperature and RS Concentration in Oysters Immediately After Processing, as Measured by the Chromate Color Test

RS, mg Cr III	Number of assays at each temperature				
	20.1°-25.0°C	15.1° -20.0° C	10.1°-15.0° C	5.6°-10.0° C	0°-5.5°C
0-0.5	1	1	15	17	3
0.5-1.0			1	4	7
1.0-1.5		1	1	2	4

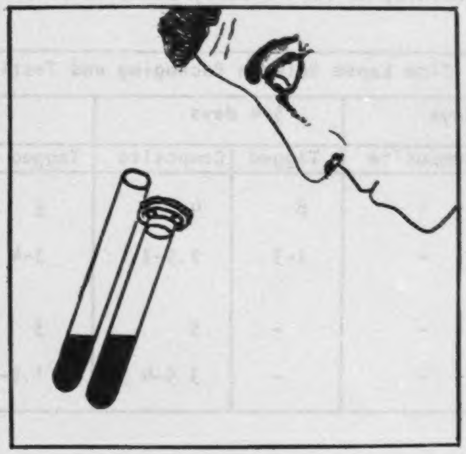

Figure 1
Reading Chromate Color Test
by
Comparison with Visual Standards

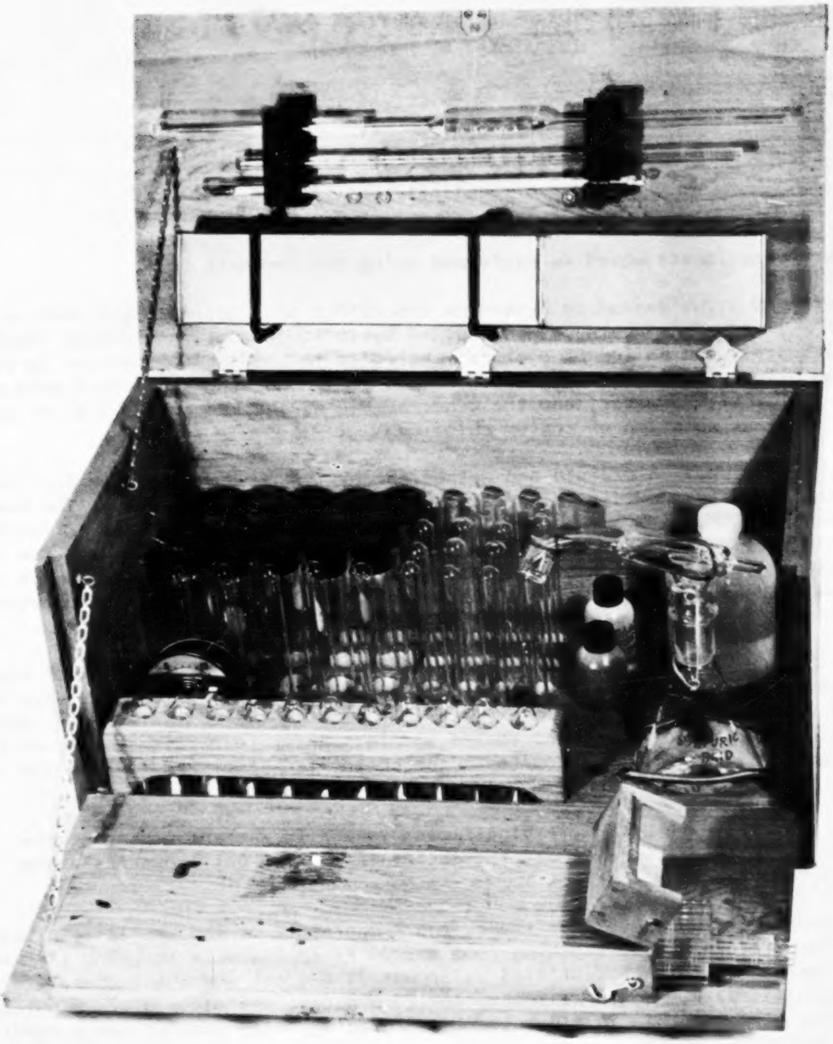

Figure 2, Field Kit for Chromate Color Test

APPENDIX P

DISCUSSION OF THE CHROMATE TEST FOR OYSTER FRESHNESS (STATEMENT OF MR. HOPE)

by

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Virginia State Department of Health
Bureau of Shellfish Sanitation

These are experiments which we performed using the chromate test.

In experiment A, we wanted to determine the effect of a saline water soak on the rate of increase of RS, reducing substance, in shucked oysters. We obtained enough shell stock to shuck approximately one gallon of oysters. We split this bushel of oysters in half, and shucked the two piles. One pile was shucked into 5-gallon holding containers with one-quarter volume of distilled water, and the other sample we shucked into a similar pot containing a 1 percent solution of sodium chloride.

After shucking we then drained and washed the oysters which were then put into six volumes of distilled water and six volumes of saline solution respectively. The temperature of the shell stock at the time of shucking was 60 degrees. We shucked them in the laboratory at a temperature of 73 degrees F. Approximate shucking time was one hour, and the temperature of the soaking solution of distilled water was 75 degrees, and for the sample was 74 degrees. We soaked these for twenty minutes and took approximately three minutes transferring them to the final pack.

Within 15 minutes of packaging the controlled sample, we had a reading of one milligram of chromate with no readings for the sample soaked in saline. After two days of storage we had a production of two milligrams of chromate in the control sample, the distilled water sample, and two in the saline soaked sample. After four days storage we had a production of three milligrams of chromate in the controlled sample and two in the saline soaked sample.

We felt that these results were significant enough to warrant repeating the experiment and this we did. We extended the period of testing until oysters became organoleptically unacceptable.

In the repeat of the above experiment, the temperature of the shell stock was 53 degrees. The temperature of the shucking room was 70°F; approximate shucking time was 1¼ hours, and the temperature of distilled water was 74 degrees and the temperature of the saline sample was 77°F. Again we soaked for 20 minutes, and allowed ten minutes draining and packaging time. Within 15 minutes after packaging, both samples had a reading of ½ milligram of chromate. After two days storage, they both read 1½ milligrams of chromate. On the fourth day of storage, the controlled sample had increased to 2½ milligrams, and the saline sample remained at 1½ milligrams of chromate. On the sixth day, the controlled sample remained at 2½, and the saline soak sample remained at 1½. On the eighth day, the controlled sample had increased to 3½, and the saline soak sample remained at 1½. On the fifth day, we had 3½ milligrams, and on the twelfth day we had a production of 4 milligrams of chromate for the controlled sample, and 3 for the saline sample. Readings were status quo for the fourteenth day and then on the sixteenth day, when the sample became unacceptable, both samples had increased to 4½ milligrams of chromate. So it seems that a saline soak retards the rate of increase of reducing substance during storage.

I have just had all my time cut off, so I'll briefly run through the remaining experiments with comments on the results and conclusions that we can draw.

Another experiment helped us draw the conclusion that the rate of increase of the reducing substance during the storage of shucked oysters is not dependent on the blowing process.

In another experiment, we determined that the temperature of the wash and soak water has no effect on subsequent rate of increase of the reducing substance. We repeated this experiment as a result of accidentally freezing the shell stock, and came to the conclusion that the storage of shell stock at either temperatures below freezing or abnormally high temperatures caused a more rapid rate of increase of reducing substance during subsequent storage.

Also if previous variables are the same, larger oysters do not produce greater amounts of reducing substance during storage. Then we performed another experiment in which we stored the oysters under atmospheric oxygen in regularly sealed containers and the conclusion was that oysters stored in open containers show a greater rate of increase than oysters stored in sealed containers. Conclusions from our final experiment indicated that the addition of salt decreased the rate of increase of reducing substance during subsequent storage.

Thank you.

APPENDIX Q

MINAMATA DISEASE

THE OUTBREAK OF A NEUROLOGIC DISORDER IN MINAMATA, JAPAN, AND ITS RELATIONSHIP TO THE INGESTION OF SEAFOOD CONTAMINATED BY MERCURIC COMPOUNDS

By

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This report is presented as an example of the need for careful study and control of possible new sources of industrial contamination of human seafood resources.

In 1953 a severe neurologic disorder was first recognized among persons living in the vicinity of Minamata Bay, Japan. Minamata City is located on the southwest coast of Kyushu, the southernmost of the main Japanese Islands, and is a few miles inland from the Shiranuhi Sea. Its population of about 40,000 people is supported mainly through employment in local industries. There is a large manufacturing plant, employing approximately 3,500 people, which has produced a variety of chemical products including nitrates, acids, and fertilizers for several decades. It began the production of vinyl chloride in 1949. The effluent from this factory was channeled into the Shiranuhi Sea until 1950, when a new channel was opened directly into nearby Minamata Bay. Although the bay is only about 10 sq. kilometers in area and is not suitable for commercial fishing, it has been used regularly as a source of seafood for many of the families inhabiting the 11 small villages along the shores of the Bay. The total population of these villages is about 10,000.

Eighty-three cases of Minamata disease in the population of the fishing villages were recorded by 1960, most ending fatally or with permanent severe disability. Epidemiologic investigations initiated by the staff of Kumamoto Prefecture University established the relationship of this illness to the consumption of seafood from Minamata Bay. The effluent from a large chemical manufacturing plant which emptied into the bay had been suspected as the source of a toxic material contaminating fish and shellfish. Earlier reports by members of the staff of Kumamoto University described the epidemiologic features of this outbreak, as well as the clinical and pathologic characteristics of the neurologic syndrome which has come to be known as Minamata disease.

In the published report in World Neurology, Vol. 1; 370, 1960, the clinical, pathologic and epidemiologic features of the disease were reviewed, evidence concerning the source and nature of the presumed toxin were presented, and the literature concerning organic mercury intoxication in man was reviewed. The possibility of additional outbreaks occurring under similar circumstances elsewhere were discussed.

Minamata disease is characterized by widespread involvement of the central nervous system with cell degeneration particularly of the granular layer of the cerebellum and lesser involvement of the basal ganglia, hypothalamus, mid-brain and cerebral cortex. The clinical observations include incoordination, involuntary movements, upper motor neuron involvement and often severe emotional and intellectual impairment. These clinical and pathological findings are similar to those of cases of the organic mercury fungicide intoxication reported by Hunter, D., and Russell, D. (J. Neurol., Neurosurg. & Psych.) 17: 235, 1954. There is essentially no difference in incidence of Minamata disease by sex, and all ages other than nursing children are involved. Cats and fish-eating birds have been affected, and fish and shellfish from Minamata Bay have produced the disease when fed to laboratory animals.

The brain, the liver, and the kidneys of patients and cats dying of Minamata disease showed elevated mercury content, particularly in cases of short duration. Early evidence suggested that an organic rather than an inorganic mercury compound was the responsible agent. The relatively high mercury content in the brain corresponded to the affinity of organic mercury compounds for the central nervous system. There was a correlation between the quantity of mercury in fish and the amount of fish required to produce the disorder in experimental animals. Small quantities (the equivalent of 20 - 80 mg. of mercury) are required to produce the disorder in cats.

Other than mercury, no toxic agent has consistently been noted in tissues of patients or experimental animals dying of this disease. Large quantities of mercury have been found in Minamata Bay and in the effluent channel of a nearby factory. Mercuric chloride is used in the catalytic process of vinyl chloride production at this plant and appreciable quantities of mercury have been lost in this process. The vinyl chloride production preceded the development of the first series of cases; a ban on fishing in Minamata Bay reduced the incidence but new cases have recurred recently presumably caused by ingestion of fish caught in nearby waters which may now be contaminated.

It is suggested that mercury combined with an alkyl radical and presumably as a complex with tissue protein may be the toxic agent in affected fish.

Inquiries in the vicinity of vinyl plants near Galveston Bay, Texas, failed to demonstrate any clinical cases. Mud and shellfish samples obtained from Galveston Bay do not show an appreciable elevation of mercury except in one swampy area adjacent to a holding basin for spent catalyst. This area is not used for fishing.

Differences in the technologic procedures of vinyl production, the varying dilution factors in Minamata Bay and Galveston Bay, the differing dietary habits in the population adjacent to the bays, and the possible ecologic variations in marine life may account for the apparent absence of clinical cases and toxic seafood in Galveston Bay.

The following recommendations regarding Minamata Bay and other areas in which similar conditions may prevail were made in the published report.

1. There is evidence that fish and shellfish from Minamata Bay are still toxic at the present time, and the ban on fishing in that area should continue to be enforced until by appropriate animal experiments, the seafood is found to be safe. Fortunately, since no commercial fishing is involved, this remains a strictly local problem in southern Japan.

2. A detailed study should be made of the ecology of the shellfish and free-swimming fish in Minamata Bay.

3. Tests should be undertaken to determine the precise chemical form in which the toxic agent exists in seafood, and the mechanism by which it is incorporated into this form.

4. A means of removing mercury-containing silt from the bottom of Minamata Bay, particularly near the effluent drainage canal where most of the mercury appears to be concentrated, seems indicated. This might require dredging the upper layers of silt in the vicinity of the old effluent channel and removing these to a safe inland storage area.

5. The possibility of employing distillation rather than washing to purify vinyl chloride should be explored. Since mercury may be a serious contaminant of air, safeguards to insure that safe levels in the air are not exceeded after distillation will also be required. If it is economically feasible, local health authorities should encourage plants not already doing so to reclaim mercury from all spent catalyst. An alternative chemical process should be sought to reduce further the use of mercury in this manufacturing procedure.

6. Further case-finding studies, epidemiologic investigations, and chemical determinations for mercury and other possible toxic agents in fish and bottom specimens are necessary in such areas as Galveston Bay, as well as in the vicinity of other vinyl plants in Japan and other countries. Fortunately, there appears to be no unsafe condition in the general Galveston Bay area.

7. The experimental study of the use of chelating agents in the earliest stages of acute intoxication in animals may provide data which would be useful in the early treatment if further cases occur.

These recommendations may aid in the control of the disease in the Minamata area, and its prevention in other similar areas.

APPENDIX R

THE OCCURRENCE OF COPPER AND ZINC IN SHELLFISH

By

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INTRODUCTION

In comparison with most other foods, marine organisms contain large amounts of copper and zinc (1). It is not unusual to find oysters with a copper content of 30 mg per kg (2) or a zinc content of 1000 to 2000 mg per kg (2, 3). In fact, according to published reports (4, 5, 6) the copper content ranges from 3 to 300 mg per kg and the zinc content from 30 to 3000 mg per kg.

INVESTIGATIONS

Because of the results of tests on the toxicity of an acid extract of oysters to mice, and the toxicity of certain whole oysters to cats, a limited survey of the zinc and copper content of East Coast shellfish was undertaken. Zinc levels well above the values cited above were found in oysters, and a wide geographic variation in both copper and zinc was noted (Figure 1). The oysters of highest zinc content (3200 to 4000 mg per kg) were found in the upper Chesapeake Bay near Baltimore and Annapolis. This is the area where Chipman et al. (7) found an unusually high zinc content in oysters (2933 mg per kg). On the other hand, oysters from the lower Chesapeake Bay near the city of Cape Charles and from the Chincoteague Bay at Chincoteague on the ocean side of the peninsula were unusually low in zinc (310 to 330 mg per kg). Oysters from the Chesapeake Bay at Deal Island, from the York River at Clay Bank, from near Shelter Island off Long Island, and from the Delaware Bay at Pierce's on Cape May had an intermediate zinc content (920 to 1900 mg per kg).

Mussels, clams and oysters differ considerably in their ability to concentrate and/or retain copper and zinc (Table 1). Comparisons of samples B-1 and C, D and E, and G and H show that the oysters contain about 10 to 20 times as much copper and 30 to 40 times as much zinc as clams from the same or nearby areas and about 200 times as much copper and 90 times as much zinc as mussels from the same area.

Oyster samples high in zinc are also high in copper, although the converse does not seem to be true. For example, sample M-1 is very high in copper but not proportionally high in zinc. In these comparisons, it was frequently noted that samples high in zinc were discolored (raw oysters had a blue-green hue). As a test about 1 pint of blue-green oysters were of normal appearance. The discolored oysters contained over 3 times as much copper but less than 2 times as much zinc as the normal-appearing oysters (see samples K-1 and K-2 in Table 1). Thus a blue-green oyster may be indicative of high copper content, but not necessarily a comparatively high zinc content.

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Since oysters lose liquor when stored, particularly after being frozen and thawed, the distribution of copper and zinc in oysters was studied to determine whether either metal was solubilized and lost in the liquor. Table II shows that little copper or zinc was found in the liquor and that the copper and zinc content of the meat appeared to increase as the liquor was lost. The effect of processing on the concentration of copper and zinc also was studied. Three samples of oysters were commercially processed; little change in their copper and zinc content was noted (Table III).

The distribution of copper and zinc in the oyster tissue was determined in about 25 drained and dissected oysters. The copper and zinc contents of the dissected parts are given in Table IV. The zinc and copper content of the abductor muscle was somewhat lower and the palps showed a lower copper content than that of the rest of the tissue, but the copper and zinc did not seem to be stored preferentially in any one tissue.

The seasonal variation in the copper and zinc content of oysters from one area of Chesapeake Bay is now being studied. As indicated in Table V, the zinc content seems to have reached a peak in August. This would indicate that the zinc content of the oyster is related either to its feeding habits or to an increase in the zinc content of the water. Increase in the zinc content of the water could result from (1) evaporation and little runoff, (2) an increase in runoff from land, and (3) an increase in industrial activity and resulting waste disposal. It would seem more likely, however that the copper and zinc content of the oyster is related to its feeding habits, since it has been shown that there is no direct relationship between the zinc content of the water and that of the oyster and that the concentration of zinc in the oyster body is thousands of times greater than that in the surrounding sea water (7). It is probable, therefore, that the oysters acquire the zinc from algae which concentrate zinc very effectively (8).

ZINC TOXICITY

Results of the tests on the toxicity of an acid extract of oysters to mice and of whole raw oysters to cats are shown in Tables VI and VII, respectively. These results raised the question as to whether the high levels of zinc in these oysters constitute a public health problem. According to McNally (3) 1 to 2 grams of zinc sulfate is an emetic dose for humans; thus the consumption of a half pint of Swan's Point oysters (Table V) might be expected to produce vomiting. Most oyster fanciers know, however, that it is unwise to eat many blue oysters, and the industry cooperates by packing few "blues" in any one container.

An excess of zinc in the diets of rats (0.5 to 1.0 percent) has been shown to cause a depression of growth, anemia, and a decrease in the activity of certain enzymes (9). The addition of copper and certain vitamins to the diet tends to overcome these adverse effects however, presence of copper in oysters that contain high concentrations of zinc may tend to offset any effect of the zinc. There is little evidence of chronic disease in humans caused by continual consumption of zinc (3). In fact, quantities of zinc varying from 10 to 200 mg per kg are present in human organs and a deficiency of zinc may cause certain diseases (10).

RELATED TOXICOLOGICAL PROBLEMS

This study has shown that oysters may accumulate large quantities of zinc, and this, with the knowledge that oysters also accumulate large quantities of paralytic shellfish poison, raises the question: What else do shellfish accumulate? In particular, do oysters accumulate the detergents or insecticides used on land and found in runoff water? Furthermore, chlorinated hydrocarbons to control oyster predators and herbicides to control aquatic plants are being used experimentally. Do oysters accumulate these compounds used directly on or over the oyster beds? At present not enough information is available to answer these questions.

ACKNOWLEDGEMENT

The assistance of Albert J. Wehby in conducting the polarographic analysis for copper and zinc is gratefully acknowledged. Recognition is also due to James E. Hanks and Royston Z. Medford (deceased), U. S. Bureau of Commercial Fisheries, Biological Laboratory, Oxford, Maryland, who arranged for the collection and shipment of the shellfish.

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Table I

Zinc and Copper Content of East Coast Shellfish

Sample No.	Kind of Shellfish	Date Collected	Location	Content,mg/kg Copper	Zinc
A	Oysters	8/24/60	Old Orchard,Tangier Sound, Md.	28.00	960.0
B-1	Oysters	8/25/60	Tollys Point,Chesapeake Bay, Md.	130.00	3200.0
B-2	Oysters	9/28/60	Tollys Point, Chesapeake Bay, Md.	230.00	3900.0
C	Clams	8/30/60	Kent Shore, Upper Chesapeake Bay	7.10	76.0
D	Mussels	9/2/60	Swan Point, Upper Chesapeake Bay	0.72	44.0
E	Oysters	3/2/60	Swan Point, Upper Chesapeake Bay	200.00	4000.0
VFL-1	Oysters	8/31/60	York River, Clay Bank, Va.	36.00	920.0
VFL-2	Oysters	8/31/60	Mouth Cherrystone Creek, Va.	8.80	330.0
F	Oysters	9/7/60	Shoal Sands, Delaware Bay	36.00	1900.0
G	Oysters	8/16/60	New Hampshire	12.00	650.0
H	Clams	8/16/60	New Hampshire	1.90	20.0
I	Oysters	9/20/60	Chincoteague,Va. Eastern Shore	16.00	310.0
J	Oysters	10/31/60	Black Rock Bar, Upper Chesapeake Bay	420.00	3300.0
K-1	Oysters	10/7/60	West Neck, Long Island	160.00	1200.0
K-2	Oysters	10/7/60	West Neck, Long Island	520.00	2000.0
L	Oysters	11/11/60	Turnagain Bay, North Carolina	30.00	390.0
M-1	Oysters	3/10/60	York River, Virginia	170.00	1000.0
M-2	Oysters	3/2/60	Egg Island, Horn Harbor	66.00	560.0

Table II

Copper and Zinc in Oyster Meat and Liquor

Portion	Cu, mg/kg	Zn, mg/kg
Meat plus Liquor	41.0	1323
Drained Meat	56.0	1657
Liquor	6.9	145

Table III

Copper and Zinc in Processed Oysters

Sample	Control		Processed	
Date	Cu, mg/kg	Zn, mg/kg	Cu, mg/kg	Zn, mg/kg
1/17/61	64	2032	63	1851
2/17/61	64	1853	69	1830
3/17/61	82	2178	60	1557

Table IV

Copper and Zinc in Oyster Parts

Parts	Percent of Total Weight	Percent of Total Cu	Percent of Total Zn
Mantle	23.1	26.0	26.9
Gills	25.7	28.6	22.8
Liver and Stomach	21.0	21.5	21.5
Palps	5.6	3.6	5.6
Abductor Muscle	8.9	3.8	4.9
Remainder	15.5	16.3	18.6

Table V

Seasonal Variations in Zinc Content of Oysters From One Area of Chesapeake Bay

	Zn, mg/kg	
Sample Date	Swan Point	Hackett Bar
1/17/61	2485	2032
2/17/61	3064	1853
3/17/61	2274	2178
4/19/61	2723	2402
5/17/61	2345	3686
6/19/61	2632	3492
7/18/61	2764	3728
8/23/61	2764	3728
8/23/61	3613	4013
9/25/61	2900	3759

Table VI

Relationship of Zinc Content of Acid Extract to Toxicity in Mice

	Content, mg/ml		Bioassay		Zinc[b]
Sample No.	Copper	Zinc	Dilution[a]	Death Time	mg/kg
A	0.0018	0.310	as is	4-24 hr	640.0
B-1	0.0110	1.460	1:3	4-24 hr	2900.0
B-2	0.0310	1.980	1:4	5-22 hr	4000.0
C	0.0025	0.020	as is	---	48.0
D	0.0007	0.008	as is	---	17.0
E	0.0210	1.960	1:4	2-24 hr	4000.0
VFL-1	0.0044	0.320	as is	4-24 hr	650.0
VFL-2	0.0017	0.190	as is	---	400.0
F	0.0036	0.780	as is	1 hr 13 min	1600.0
G	0.0020	0.230	as is	---	470.0
H	0.0014	0.010	as is	---	21.0
I	0.0026	0.130	as is	---	270.0
J	0.0430	1.560	1:3	4-20 hr	3100.0
K-1	0.0091	0.590	as is	2 hr 56 min	1200.0
K-2[c]	0.0650	1.040	1:2	6-22 hr	2100.0
L	0.0036	0.190	as is	---	370.0
M-1	0.0054	0.450	as is	---	900.0
M-2	0.0038	0.270	as is	---	560.0

[a] Largest dilution causing death of at least two out of three mice (1 ml injected).

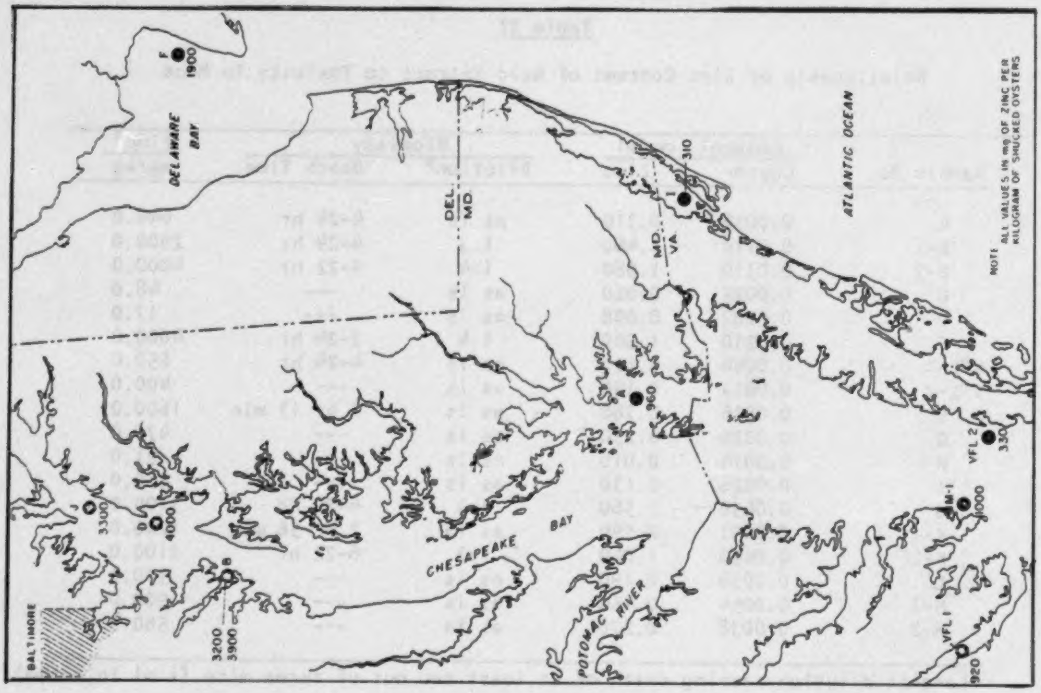
[b] Assuming an equal distribution of the zinc between the meat and the liquid extract. (Compare with values in Table III). Copper is not similarly distributed; only about 10 percent is found in the extract.

[c] About one pint of discolored (blue-green) oysters picked from one gallon of Sample K.

Table VII

Oral Feeding of Whole Raw Oysters to Cats

Sample	Amount Eaten Oysters g	Amount Eaten Zinc mg	Weight of Cat, g	mg Zinc per kg Animal	Time before Vomiting, hr
A	252	241	3600	67	-----
A	148	142	3050	46	-----
B	54	173	1250	138	4
B	112	358	3250	110	2 1/2
B	205	656	3650	180	1 1/2


F
1900
DELAWARE BAY
DEL.
MD
MD.
VA.
310
ATLANTIC OCEAN
960
VFL 2
330
M-1
1000
VFL 1
920
POTOMAC RIVER
CHESAPEAKE BAY
BALTIMORE
3200
3900
B
C
3300
D
4000

NOTE: ALL VALUES IN mg OF ZINC PER KILOGRAM OF SHUCKED OYSTERS

GEOGRAPHICAL VARIATION IN ZINC CONTENT OF OYSTERS
FIGURE I

APPENDIX S

PROGRESS IN DEVELOPMENT OF METHODS OF CHEMICAL CONTROL OF SHELLFISH PREDATORS

by

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U. S. Bureau of Commercial Fisheries
Biological Laboratory, Milford, Connecticut

BULLETIN NO. 3

In accordance with our practice of keeping marine biologists and members of shellfisheries industries informed of our activities which have direct practical application, we are issuing this bulletin with new information on our progress in developing and testing the chemical methods of control of shellfish enemies. It contains our report, which we presented at the convention of the National Shellfisheries Association last week, on field tests of the method for control of boring snails. Its purpose is to bring this information to those people who were unable to attend the convention.

We are also taking this opportunity to inform interested persons as to the steps that are being taken to clear the method for industrial use. It will be remembered that in all our previous publications, including bulletins, it was strongly emphasized that although the method is quite effective against boring snails, such as oyster drills, and several other molluscan enemies, its use is not recommended until we determine the effects of the chemicals constituting our formula on water communities in general, and also ascertain whether some of these substances accumulate in the body tissues of mollusks, such as oysters and clams, rendering them undesirable or dangerous as food.

In accordance with this principle, representatives of the Bureau of Commercial Fisheries and other Federal institutions, including the United States Public Health Service and Food and Drug Administration, held several meetings to decide the steps that should be taken to determine the safety of the method. Since then, to test the method, a number of experimental oyster plots in Long Island Sound have been treated with different formulae and different quantities of the chemicals. At present, samples of oysters and clams are collected at definite intervals from these plots and adjacent areas to be tested for traces of the chemicals in their meats.

Because accurate testing methods have already been developed and approved for Sevin, one of the components of our formula, tests for residues of this chemical in molluscan meats are already in progress. However, since no methods were available for determining small quantities of chlorinated benzenes, such as orthodichlorobenzene, in tissues of clams and oysters, a contract to develop such a method has been signed with the U. S. Testing Company, Inc. of Hoboken, New Jersey. Only after a method has been developed and accepted by proper authorities, which may take several months, can analysis of the samples begin. We are now collecting samples, however, that will be ready for analysis as soon as a method is developed.

We are again asking oystermen not to employ the method until it is approved since improper use of it may lead to many undesirable misunderstandings and mistakes. Meanwhile, we shall continue to develop various aspects of the method in an effort to make it more versatile, effective, cheaper and, most of all, safer for aquatic communities.

FIELD TESTS OF A CHEMICAL METHOD FOR THE CONTROL OF MARINE GASTROPODS

by

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and
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Introduction

As a result of several years of work in screening various chemicals to develop methods to control oyster enemies, Loosanoff, MacKenzie and Shearer (1959, 1960) found that certain high density organic solvents, the polychlorinated benzenes, either alone or in combination with certain other compounds, anesthetized gastropods in a relaxed and expanded state and recommended the use of such chemicals in the control of predators of commercial mollusks. Later, Loosanoff, MacKenzie and Davis (1960) and Loosanoff (1961) reported further laboratory and preliminary field tests on the effectiveness of these chemicals mixed with dry sand for the control of shellfish predators, chiefly boring gastropods and starfish.

During the summer of 1960 new and more extensive field tests were conducted, using one of the basic formulae designed to control oyster drills and other gastropods. The purpose of these tests was fourfold: (1) to evaluate several methods of application of chemically-treated sand; (2) to determine the effectiveness of several variations of the method in the control of oyster drills and other gastropods; (3) to determine the effect of treatment on other fauna; and (4) to determine the effect of treatment on setting of oysters and starfish in treated areas.

Materials and Methods

Tests were confined to the use of solutions of Sevin in orthodichlorobenzene (to be referred to as O.D. in this article), which were then mixed with sand as an inert carrier. Two concentrations of Sevin were used, a solution containing 0.7 percent by weight of Sevin in O.D. (4.2 lbs. of Sevin per 55-gallon drum of O.D.) and a solution containing 2.1 percent by weight of Sevin in O.D. (12.6 lbs. of Sevin per drum of O.D.). In all tests the ratio of these chemicals to sand was ten gallons of the solution to 1 cubic yard of sand, i.e., approximately a 1 to 19 ratio by weight. On all except two plots we used sand that had been kiln-dried, cooled, and stored dry until mixed with the chemicals. Applications of 1, 3, 5 cu. yds. per acre were used.

Two methods of spreading the sand were tested. The first consisted of distributing the treated sand overboard by means of a spreader, of the type employed by highway departments, mounted on the bow of the boat. The second method consisted of washing sand overboard by a stream of water from a high pressure pump, with which many of the oyster boats in the Long Island Sound area are already equipped.

A team of SCUBA divers, under the direction of Mr. Clyde L. MacKenzie, Jr., observed the underwater spreading of the sand, the degree of coverage of the experimental plots, and the effects of the treatment on the pelagic and bottom fauna. In areas where the water was of sufficient depth, the divers made observations while the treated sand was actually being spread, but in shallower water, where this would have been hazardous for the divers, their observations were made immediately after the treatment. The divers also made follow-up examinations of the plots at certain intervals after the treatments.

To determine the effect of treatment on setting of oysters and starfish thirteen additional stations, comparable to our ten regular stations for observing setting, were established on and adjacent to treated areas (Figs. 4, 5 and 6).

Experimental Results

The tests, in which we used sand that was not kiln-dried, proved to be less satisfactory than those using thoroughly dry sand. When damp sand directly from commercial stockpiles was used, much of the chemical solution drained out of the sand onto the deck of the boat in a short time, thus reducing the effective concentration before it was even put overboard. Moreover, damp sand loses more of the chemicals than dry sand as it descends through the water. Nevertheless, on the test plot, on Lot 683 in the Milford area of Long Island Sound receiving five yards per acre of non-dried sand treated with a 2.1 percent solution of Sevin in O.D., the divers reported Polinices and eventually Busycon were affected. Moreover, the 25 healthy oyster drills, Urosalpinx cinerea, placed on the plot the following day became partially swollen within 30 minutes. The divers were of the opinion, however, that the treatment was either less effective or lost effectiveness more rapidly than other plots receiving equivalent quantities of treated dry sand. This may have been in part due to heavier siltation or stronger currents in this particular area.

The second plot, treated with non-dried sand, O.D. and Sevin, was off the east end of Long Island in deep water and with a fairly strong current. In this experiment apparently very little treated sand actually reached the bottom on the test plot and the treatment was almost wholly ineffective. This was the result of a combination of factors including, in addition to the use of non-dried sand, an ineffective spreading pattern due to fog, deep water, and strong currents.

So far as could be determined, there was no difference in the effectiveness of the treatment, whether the sand was washed overboard with strong streams of water or when a sand spreader was used. In both cases the sand is spread over the surface and disperses relatively uniformly as it descends through the water so that, in the course of several passages over an area, the bottom is thoroughly covered. Divers describe the falling sand as looking like "snowflakes descending in a heavy snowstorm."

Washing the sand overboard (Fig. 1) is much easier for the crew than the mechanical method of spreading because there is less handling of the sand and no dust. In either case, however, since the sand spread on the surface may have to fall through 8 to 30 feet of water, the precision with which sand can be placed on a designated area depends upon the depth of the water and the strength of the current. Thus, either method will give good coverage of an area in shallow water or when there is no current. In deep water, however, even a slight current can cause drifting which will scatter the sand over a wider area than intended, resulting in an insufficient coverage to be effective. Evidence of such drifting of the falling sand with the current was observed in the treatment of Lot 636 in the Milford area and, as previously mentioned, was probably a major factor in the failure of the test off the end of Long Island.

A device for spreading the sand within a foot or so of the bottom would have several advantages and is the only practical method for treating bottoms in deep water or in water where there is always strong current. The disadvantage of such a method of spreading is that it is virtually impossible to get complete coverage, i.e., small "islands" will usually be left untreated.

The softness of the bottom and the extent of siltation are also conditions influencing the effectiveness of area treatment with chemically impregnated sand. In one test, in the Thimble Islands district of Long Island Sound, where the bottom was extremely soft, the sand either sank into the mud or was so quickly covered by silt that the treatment had little effect. In another test, on the inshore plots in the Milford area on Lots 639 and 683, the divers reported that within a few days the sand was covered to such an extent that it was no longer visible, but it could be felt when the silt was rubbed between the fingers and gastropods were still strongly affected. Under such conditions, however, the treatment almost certainly loses effectiveness more rapidly.

The effects of treatment on pelagic and bottom-dwelling fauna also differed somewhat with the area, depth and currents. Thus, in the open waters of Long Island Sound the divers found no evidence of any effect on bony fish and only an occasional hermit crab or mud crab showed some evidence of irritation. In New Haven Harbor in shallower, quieter water, they found one pipefish apparently killed along with some crabs and small razor clams. Finally, in the very shallow, quiet water of Great South Bay, several small fish that were in the area as the sand was descending, as well as crabs and jellyfish, were killed. On all of our tests plots, however, all types of fish and crabs were observed feeding and moving normally within a few days after treatment. In fact, in several instances the divers reported fish to be more numerous a day or so after treatment of an area, apparently being attracted by the swollen, dying gastropods. On all treated plots small pelagic, shrimp-like crustaceans, present in the area as the sand was descending, were killed, but again this effect was only during the period of treatment. Once the treated sand was on the bottom it had no further effect on the pelagic crustaceans nor on bottom-dwelling crabs.

In an area where oysters and mussels were present the divers found them open and pumping normally when first observed within an hour or so after a treatment, consisting of five yards of the sand per acre, had been completed.

Starfish on an area receiving treatment were irritated by the treated sand particles which lodged among the spines on the aboral surface and adhered there. Divers reported that starfish, which initially were inactive on the bottom, began to move quite actively when hit by the treated sand and soon tended to curl their rays so that some of them were easily rolled along by the current.

Examination of the treated plots a day or so after treatment showed many stars with burned areas over the aboral surface. In most instances, however, these injuries were not sufficient to kill the starfish. On areas receiving a light treatment starfish were so little affected that they attacked the swollen gastropods immediately. In heavier treatments the starfish were irritated sufficiently that it was a day or so before they would feed. Moreover, a heavy treatment tended to keep starfish out, at least for a short time. Divers reported fewer starfish on heavily treated than on adjacent control plots. It should be mentioned here that the chemical formulae used in these cases did not contain special anti-starfish compounds.

Gastropods of all kinds, including Eupleura, Urosalpinx, Polinices, Nassa and Busycon, were strongly affected (Figs. 2 and 3). Particularly on plots receiving five yards per acre of treated sand, nearly all snails of the "hundreds and hundreds" observed were greatly swollen and "lying on their backs" with the swollen foot extended. In this position they are rapidly attacked by fish, crabs and starfish.

In some cases almost every swollen Polinices had a starfish wrapped around it and, within two days, most of the gastropod shells were completely cleaned out. Those with flesh remaining inside showed evidence of being partially eaten by fish or crabs. On each of the plots a few gastropods were found, inside "boxes" or under shells, that had been protected and were not swollen. Even several days after a treatment, however, healthy oyster drills taken down and placed on a treated plot became swollen within 30 minutes. Any gastropods protected during treatment, therefore, would become affected as soon as they attempted to move about.

Probably the most effective treatment was that in Great South Bay, undertaken upon the request of the Bluepoints Oyster Company. This plot received five yards per acre of sand treated with O.D. containing 2.1 percent of Sevin. This was the heaviest of the treatments tried last summer and was in an area of shallow, quiet water. As a result, the entire area was uniformly covered with a layer of sand almost 1/16 inch thick. In this area, in addition to killing several small bony fish as the sand descended, the treatment carried jellyfish to the bottom, caused tunicates to contract, and killed several mud and hermit crabs. Spider crabs, however, did not appear to be affected. Oyster drills, Nassa and Busycon, were all greatly swollen just as they are in laboratory tests. This included several oyster drills brought up by the divers on large clusters of oyster shells and spat, even though these drills must have been two to three inches above the bottom where they might normally be expected to escape the treatment.

The effectiveness of this treatment was followed by personnel of the Bluepoints Oyster Company, who trapped for drills on the treated area and on an adjacent untreated area. Their results indicated that the treatment markedly decreased the drill population and that it remained low throughout the summer. This area was treated on July 16. On August 18 they found an average of one drill per trap on the treated area compared to 16 per trap on the untreated area. On August 26 the average was one drill per trap on the treated area to 12 per trap on the untreated area, and by September 6 it was 0.25 drills per trap on the treated area to 23.5 per trap on the untreated area.

Treatment of an area appeared to have no effect on the setting of oysters or starfish in Long Island Sound. Data on setting of oysters and starfish on treated and untreated areas are summarized in Figures 5 and 6, which show the number of oysters and starfish setting, per hundred shells, at each of the stations during the summer of 1960. A study of Figure 5 shows the setting of oysters decreasing progressively as we go away from shore and, also, as we go from west to east with no interruption of this pattern of setting, even though some stations were on treated plots and some were not. The two most inshore stations had by far the heaviest oyster set: 2,830 at our Station #2 on an untreated area, and 4,600 at Station #23 on a plot which received five yards per acre of sand treated with a solution containing 2.1 percent of Sevin in O.D., the heaviest rate used in our tests. In New Haven Harbor, on Lot 13, where each plot received three yards per acre of sand treated with 0.7 percent Sevin in O.D., and on adjacent untreated areas there was much less of a pattern to the setting, but again there was no indication that the treatment interfered with setting of oysters (Fig. 6).

Setting of starfish was considerably heavier at the deep water stations, #3 and #7, than at the more inshore stations and setting of starfish inside New Haven Harbor was considerably lighter than just outside the Harbor or in the Milford area. Within the immediate vicinity of the experimental plots, on Lot 13 in New Haven Harbor, there seemed to be no detectable pattern, and if the treatment had any effect on setting of starfish in this area, it was not apparent from our station counts. In the Milford area, also, there appeared to be no pattern to the setting of starfish, and again it did not appear that the treatments had affected the intensity of setting of starfish. It may be significant, however, that Station #21, on a plot treated July 12 just prior to the peak of starfish setting, had a very light starfish set. The plot on which Station #23 was located was also treated in mid-July but starfish setting did not seem to have been reduced here. We had no control station near enough, however, to accurately estimate the number of starfish that would have set had the lot not been treated.

Discussion

The use of dry sand treated with O.D. and Sevin, as described here, is a potent tool for the control of gastropods but has little effect on other members of the ecosystem. Although some burrowing worms, crustaceans and, perhaps, other species may be killed in the treated areas, it should be remembered that, even if all commercial oyster beds were treated, there would still be vast areas untreated where these organisms would continue to thrive. Use of the method on oyster beds, therefore, could not seriously upset "the balance of nature" in the area as a whole.

It should also be kept in mind that this is primarily a method of control, not of complete eradication. Since the treatment affects only the two dimensional areas of the bottom actually covered by treated sand, even the gastropods against which it is so effective would not be eradicated, except in small bodies of water where the entire bottom area could be covered. Predation by the gastropods remaining in untreated areas would, therefore, still serve to limit the increase of barnacles, mussels and other competing forms that might occur if complete eradication were effected.

The authors wish to express their appreciation to Mr. J. R. Nelson and the F. Mansfield & Sons Oyster Company, and to Mr. E. W. Usinger and the Bluepoints Oyster Company, for their generous contribution of time, equipment, and grounds for these tests. The help of the State Shell Fish Commission on numerous occasions is also acknowledged with gratitude. Special thanks are due to Mr. J. F. Lucash of our staff for his untiring efforts, ingenuity, and initiative in solving the many problems involved in preparing and spreading the treated sand.

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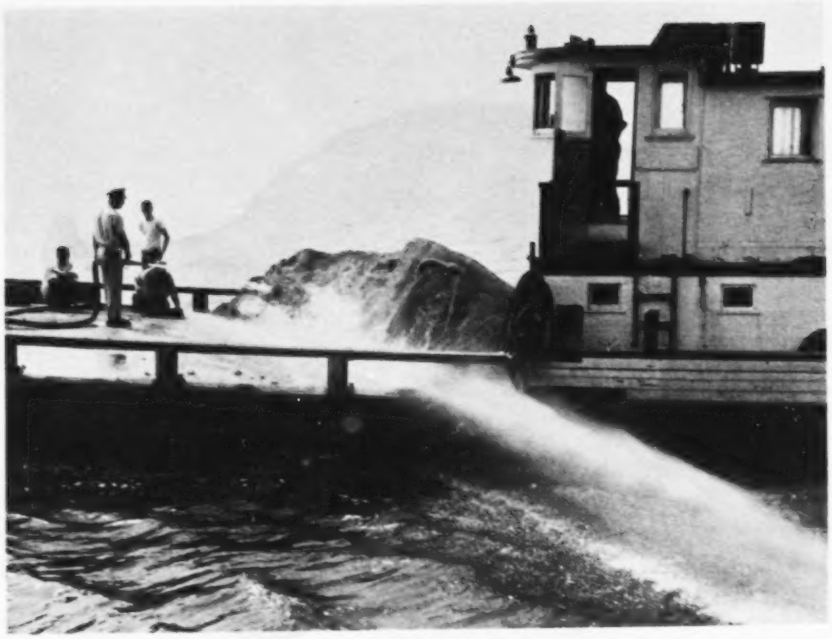

Figure 1. Method of spreading treated sand using a stream of water from a high pressure pump.

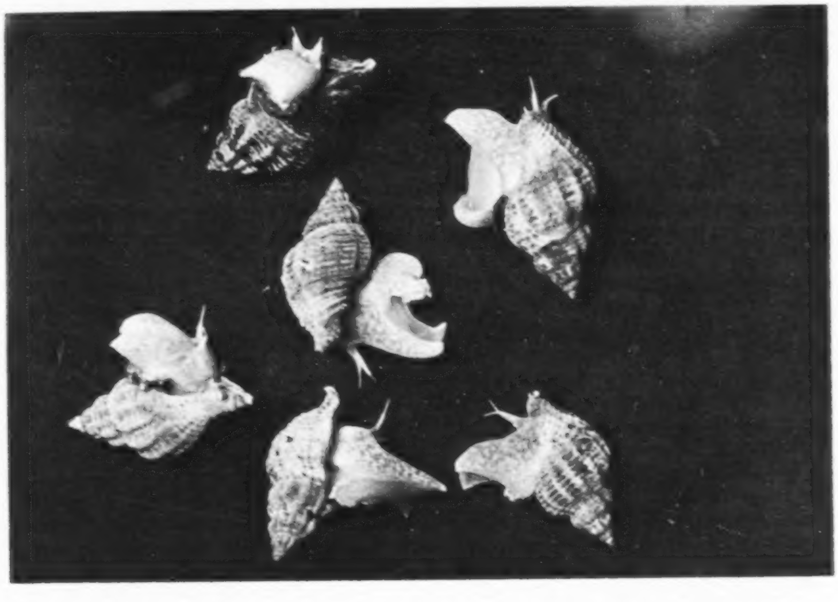

Figure 2. Oyster drills, paralyzed by the chemicals used in the treatment, with the swollen foot extended.

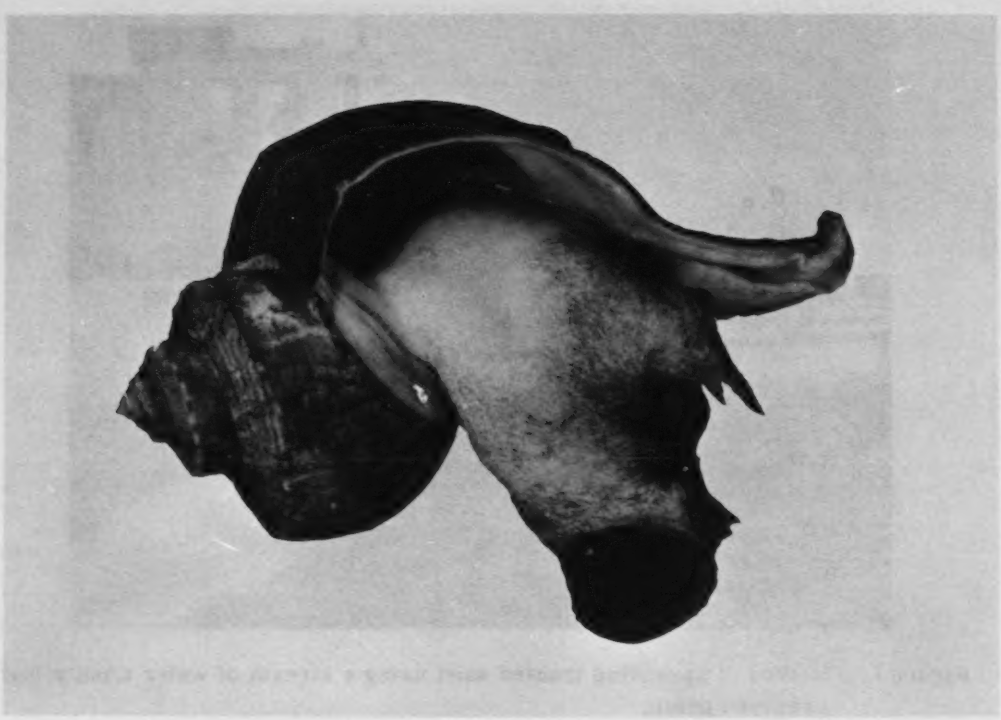

Figure 3. A conch, Busycon, paralyzed by the chemicals used in the treatment, with the swollen foot extended.

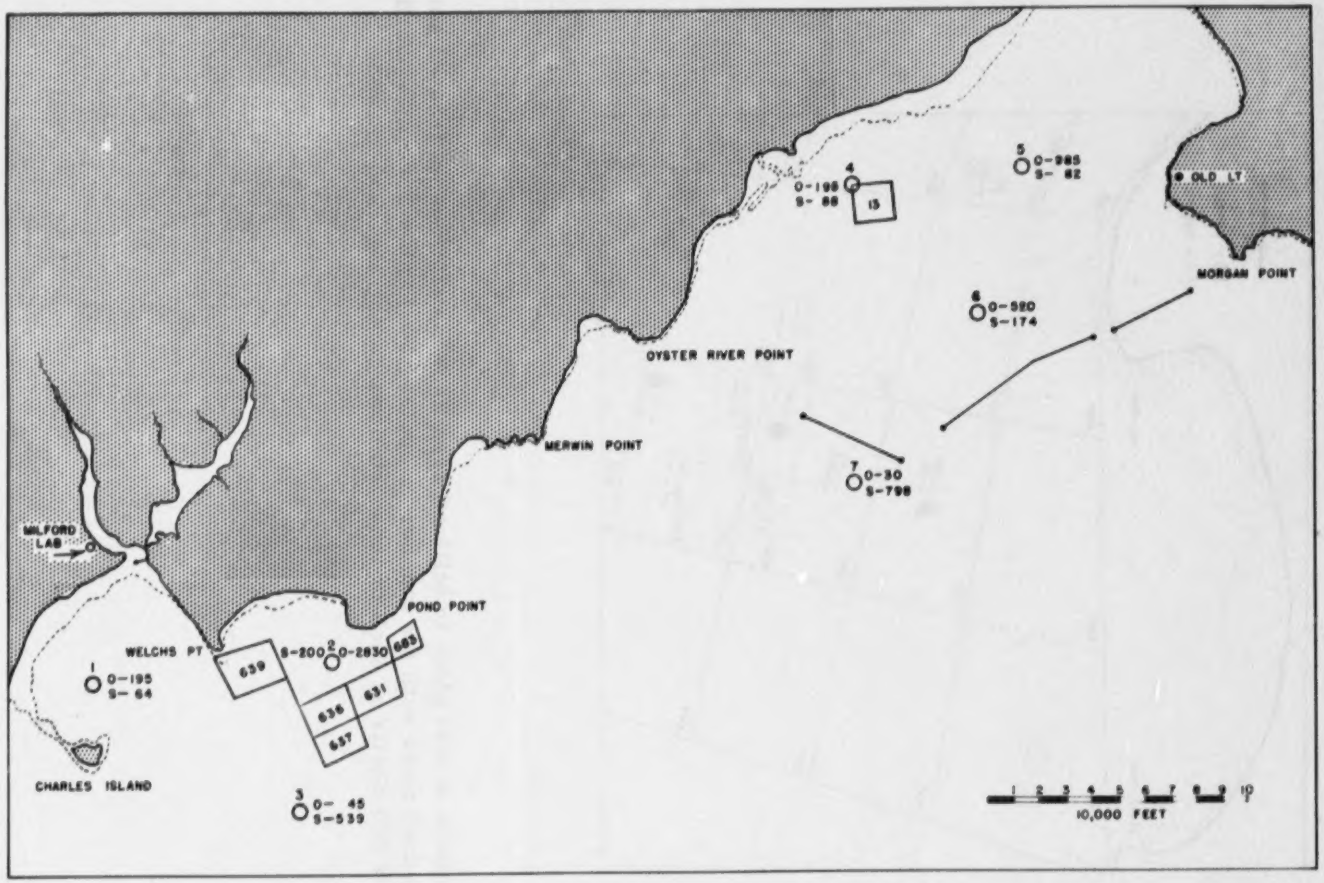
MILFORD LAB
WELCHS PT
CHARLES ISLAND
POND POINT
MERWIN POINT
OYSTER RIVER POINT
MORGAN POINT
OLD LT
1 O-195 S- 64
2 S-200 O-2830
3 O- 45 S-539
4 O-198 S- 88
5 O-285 S- 82
6 O-520 S-174
7 O-30 S-798
639
636
637
631
683
15
1 2 3 4 5 6 7 8 9 10
10,000 FEET

Figure 4. Milford-New Haven area of Long Island Sound showing location of oyster grounds used in field tests, and location of regular stations for studying intensity of setting of oysters and starfish. The number of oysters (O-) and starfish (S-) setting per 100 shells at each of these stations during the 1960 season is shown.

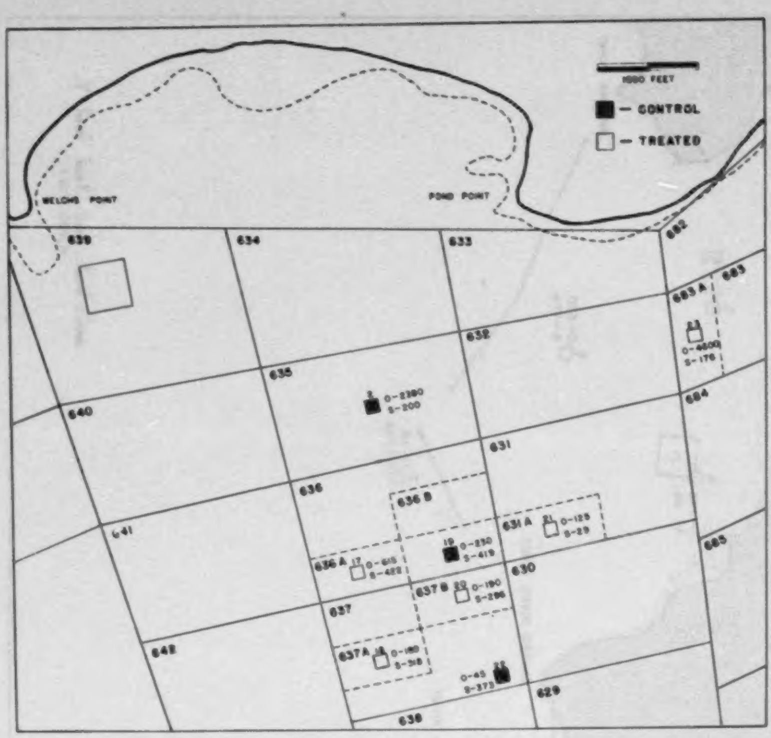
1000 FEET
■ — CONTROL
□ — TREATED
WELCHS POINT
POND POINT
639
634
633
682
683
683 A
0-4600
S-176
632
635
0-2390
S-200
640
684
631
636
636 B
631 A
21
0-125
S-29
19
0-230
S-419
641
636 A
17
0-615
S-422
630
685
637 B
20
0-190
S-296
637
642
637 A
0-180
S-318
0-45
S-373
629
638

Figure 5. Details of test plots in Milford area showing stations on treated and control plots with the number of oysters (O-) and starfish (S-) setting per 100 shells at each of these stations during the 1960 season.

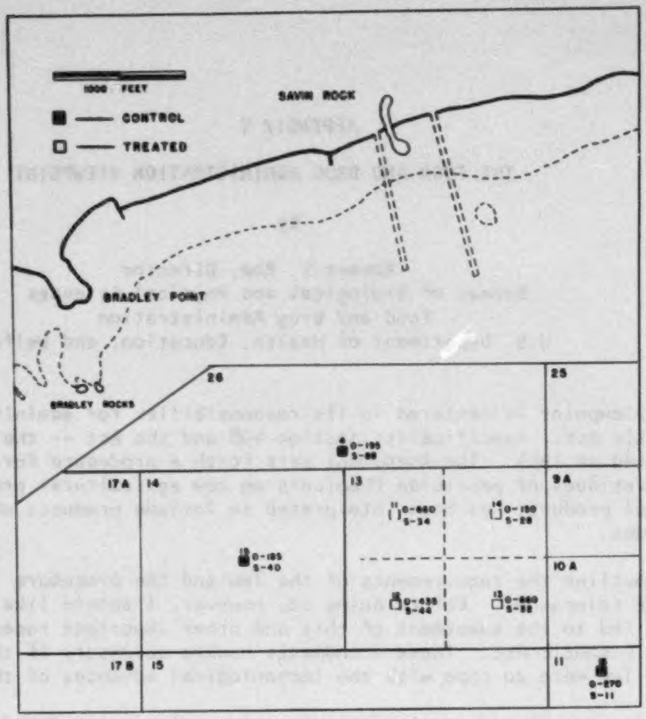
1000 FEET
SAVIN ROCK
CONTROL
TREATED
BRADLEY POINT
BRADLEY ROCKS
26
25
17 A
14
13
9 A
10 A
17 B
15
11
0-195
S-88
0-660
S-34
0-150
S-28
0-195
S-40
0-435
S-44
0-660
S-88
0-270
S-11

Figure 6. **Details of test plots in New Haven Harbor showing stations on treated and control plots with the number of oysters (O-) and starfish (S-) setting per 100 shells at each of these stations during the 1960 season.**

APPENDIX T

THE FOOD AND DRUG ADMINISTRATION VIEWPOINT

By

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U.S. Department of Health, Education, and Welfare

The FDA viewpoint is centered in its responsibility for administration of the Food, Drug, and Cosmetic Act. Specifically, section 408 and the Act -- the Pesticide Chemicals Amendment, enacted in 1954. The Amendment sets forth a procedure for establishing safe tolerances for residues of pesticide chemicals on raw agricultural products. The term "raw agricultural product" has been interpreted to include products of the sea such as fish, oysters, and clams.

I shall outline the requirements of the law and the procedure involved in the establishment of tolerances. Before doing so, however, I should like to recall some of the background that led to the enactment of this and other important recent amendments to the Food, Drug, and Cosmetic Act. These amendments became necessary if the consumer protection intended by the law were to cope with the technological advances of this scientific era.

The rapid and profound developments in science in recent decades have hazardous as well as happy implications for our society and civilization. They have had significant and important applications in the area of our responsibility -- the regulation of foods, drugs, and cosmetics. The host of new organic chemicals -- beginning with DDT -- that have appeared since the war have found application in agriculture and industry in food production as pesticide chemicals, as food additives to accomplish some real or fancied improvement, as ingredients of packaging materials or food-handling equipment.

Legislative bodies have studied the public health implications of our changing environment. In 1950 the House of Representatives of the Congress established the "Select Committee to Investigate the Use of Chemicals in Food Products," under the chairmanship of Mr. Delaney. It held extensive hearings over a period of 2 years and made recommendations for legislation. The Congress has followed up with enactment of the Pesticide Chemicals Amendment of 1954, the Food Additives Amendment of 1958, and the Color Additive Amendments of 1960.

The governors of Wisconsin and California recently appointed committees of scientists to study the public health implications of scientific developments, particularly the increasing utilization of chemicals as food and feed additives and as agents for control of pests in production of food crops.

Many scientists and scientific societies have directed attention to these problems of the atomic age. The reports of the "Interim Committee on the Social Aspects of Science" of the American Association for Advancement of Science are particularly pertinent.

Committees of the National Research Council of the National Academy of Sciences have considered and issued reports on various aspects of chemical additives and pesticide chemicals.

There is general recognition in the scientific community of the potential public health hazard in the widespread use of a multitude of chemicals in ways that might leave harmful residues on or in food products. It is the stated purpose of the recent amendments to place upon proponents of the use of chemicals the responsibility to establish safety of proposed uses. (Previously the burden was on the Government to prove a chemical harmful before its use in food could be prevented.)

The Pesticide Amendment provides a procedure for setting safe tolerances for residues of pesticide chemicals, or for the establishment of exemptions from the necessity of tolerances when such are not necessary to protect the public health. Any person who has registered or who has submitted an application for the registration of an "economic poison" under the Federal Insecticide, Fungicide, and Rodenticide Act in the Department of Agriculture may file with the Department of Health, Education, and Welfare a petition proposing a tolerance or an exemption. The petition must be supported by certain information including the identity and composition of the pesticide chemical, methods for analysis, complete information on the use, full reports of investigations made with respect to residues resulting, and of course full information on the toxicity of the chemical.

The applicant also submits a copy of the petition to the Department of Agriculture. When that Department issues a certificate of usefulness, then the Department of Health, Education, and Welfare has 90 days to act upon the petition.

Provision is made for referral of the petition to an advisory committee if either the petitioner or the Secretary desires such referral within the 90-day period. After publication of an order, any person adversely affected may file objections and request a public hearing. Finally, provision is made for court review under certain circumstances involving an actual controversy.

When the use of a pesticide chemical does not result in a residue in a food crop at the time of harvest, there is no need for a tolerance and no problem exists so far as the residue requirements of the FD&C Act are concerned, but great care must be exercised in the use of pesticides on a no-residue basis to be sure that there are, in fact, no residues. This calls for carefully controlled experimentation and examination of samples of crops by methods of analysis that have been validated and that can be relied upon for accurate information.

There are many technical and scientific problems in setting safe tolerances. First, are we sure of the identity and composition of the pesticide chemical? Is the "chemistry" in the petition adequate? Are the residues the same chemical or have there been changes, such as oxidation? If the chemical is absorbed or ingested by the crop -- the organism such as oysters -- are there metabolic changes and, if so, what are the metabolites? Are the analytical methods adequate to detect and measure any residues? Are the toxicity data adequate to enable an evaluation of the safety of the residues? To be meaningful, these data must reflect the toxicity of the residues, which may be different than the chemical applied if there have been chemical changes.

In establishing safe tolerances, we are concerned primarily with chronic toxicity. We are not ordinarily dealing with considerations of acute toxicity. We are aiming to set tolerances that are safe, assuming the ingestion of the tolerated residues more or less regularly over long periods of time -- perhaps a lifetime. You who are working in the field may well have concern also for the potentials of acute toxicity, and that certainly is a very real problem with many of the very potent poisons in use today.

Usually the toxicity data should include long-term feeding tests on two species of laboratory animals. Other tests may be appropriate for some types of compounds such as cholinesterase inhibition of organic phosphate compounds.

To sum up, the FDA is interested in the Fish and Wildlife Service research on the use of chemicals in control of shellfish predators. We are interested in all scientific developments in production of food crops. This is a particularly intriguing use of chemicals and obviously of important practical application if successful. It is essential to know whether such use results in residues of chemicals in the food products of the sea and, if so, what the residues are and whether they are safe.

APPENDIX V

STATUS OF PARALYTIC SHELLFISH-POISONING RESEARCH IN CANADA

By

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In Canada, cases of paralytic shellfish poisoning from consumption of clams and mussels have been reported from British Columbia on the Pacific coast and the estuary of the St. Lawrence and the Bay of Fundy on the Atlantic coast. Since 1943, the management of shellfish toxicity problem has been a joint concern of the Fisheries Research Board of Canada, the federal Department of Fisheries and the Department of National Health and Welfare. Based on year-round sampling of shellfish from toxic areas and assaying for poison levels, a control and management scheme has been worked out which is operative on both the Atlantic and Pacific coasts.

Our understanding of the poison problem is much better in the Bay of Fundy than anywhere else in Canada. This is because in the Bay of Fundy shellfish toxicity is an annual recurrence during summer months and we have fairly satisfactory information on the annual and seasonal variations in toxicity levels recorded since 1943. The efficacy of the existing control and management system came under a critical scrutiny after 1957 when serious outbreaks of poisoning occurred on both the Atlantic and the Pacific coasts. Since then an urgent need has been recognized for a better system of control which should enable us to make long-range predictions for each summer of, (1) the time when the shellfish become toxic, and (2) the toxicity levels to be expected.

From time to time it has been pointed out that until basic biological research is carried out it will be difficult to understand the poison problem. In this connection we need to determine (1) the source of poison, (2) the factors that regulate its occurrence and amount, and (3) which of these factors deserve field and/or laboratory investigation.

Sufficient indirect and circumstantial evidence has accumulated to implicate photosynthetic marine dinoflagellates of the genus Gonyaulax as the source of toxicity in clams and mussels. Recent experimental work by Burke et al (1960), Murtha (1960) and other workers at the U.S. Army Chemical Warfare Laboratories has established chemical, physical, pharmacological similarities between toxin derived from Gonyaulax and that found in shellfish. Today we are more convinced than ever that it is in dinoflagellates that we should look for the real source of poison. The simultaneous appearances of high toxicities and large numbers of G. tamarensis in plankton samples from the Bay of Fundy lead us to believe that these dinoflagellates are the ultimate source of toxin in shellfish (Medcof, et al, 1947; Needler, 1949).

From our experience in Canada, it has become increasingly clear that in order to perfect the existing system of control and management of the toxicity problem we must first understand the intricate relationships between the environmental conditions and the causative organism and try to isolate those conditions which favour its appearance and reproduction and those that bring an end to each season's flowering. Efforts to examine these relationships were begun in January 1961.

The most complete records of shellfish toxicity on the Atlantic coast are those for mussels from Head Harbour, New Brunswick, which has also been regarded as a "key station" the entire Bay of Fundy. Fortunately we also have a complete record of hydrographic conditions in this general area for the last 30 years. We have examined fluctuations in seasonal and annual levels of toxicity in relation to some hydrographic and meteorological conditions and have discovered interesting correlations. A fuller statement on the results of this analysis is appearing in print very shortly (Prakash and Medcof, 1961). The tentative conclusions from this study are being used as guides in planning field and laboratory studies of the peculiarities of the life history of the causative organism and we hope that they may stimulate similar investigations in other affected areas. Finally it is our hope that such an examination would provide information on the merits of various environmental indices on which long-range predictions of toxicity levels might be based.

Environmental factors

The time when poison appears in any particular year seems to depend on the warming of the water to some undetermined threshold temperature. This occurs in summer and may be as early as June and as late as September. Clarification of this relationship is being attempted. At Head Harbour, which is affected the year round, the peak toxicity scores are reached in August and September and this is coincident with rapidly increasing summer water temperature.

On a between-year basis there is no tendency towards high toxicity scores in years with warmer summers or vice versa. These observations suggest that high temperature may play an important part in stimulating toxin accumulation but the level of toxicity in any particular year is probably governed by other factors. In this connection the relation between winter water temperature and toxicity levels in summer following appears significant (Bond, 1958).

Seasonal and annual variations in river discharge appear to affect abundance of the causative organism and hence levels of toxicity at Head Harbour. At present it is extremely difficult to isolate the results of river discharge, i.e., introduction of nutrients, salinity changes, flushing action, and reduction of illumination through turbidity due to silt and detritus. Critical experimental work is needed to determine how these factors, either operating along or in combination, affect population abundance of the causative dinoflagellates.

Another important factor is the quantity and quality of light penetrating the surface layers. At Head Harbour, duration of sunshine before the onset of the toxicity period appears significant, probably by its effect on the photosynthetic activities of causative dinoflagellates. Certain biological factors, chiefly predation by other plankters, are also important. The abundance of *Gonyaulax tamarensis*, the suspected species in the Bay of Fundy, is shown to be affected by a species of ciliate which feeds on it (Needler, 1949).

Current investigations and future plans

Our year-round plankton survey at Head Harbour, N.B., has shown quite conclusively that the abundance of *Gonyaulax* coincides with the rise in shellfish toxicity as well as poison content in plankton extracts. We also find that different species of clams from the same beds store different amounts of poison. Bar clams, for instance, store five to ten times as much poison as soft-shell clams. Experiments are in progress to determine how the poison content of various species of shellfish is affected by holding them alive in filtered sea water.

We are continuing and extending these fruitful lines to study. Next year we hope to intensify our plankton reconnaissance by systematic year-round collection from affected areas in the St. Lawrence estuary as well as in the Bay of Fundy.

Early this year our efforts were largely confine to isolating the suspected dinoflagellates from Bay of Fundy plankton and trying to culture them in the laboratory. We havesucceeded only recently in establishing and maintaining healthy cultures of Fundy Gonyaulax in artificial media. We hope to work out laboratory mass culture techniques soon and direct our researches along specialized lines. We would like to know the thermal, photic and nutritive requirements of these organisms; to do bioassay of toxins extracted from pure cultures and to observe how toxicity levels are affected when shellfish are fed on dinoflagellates.

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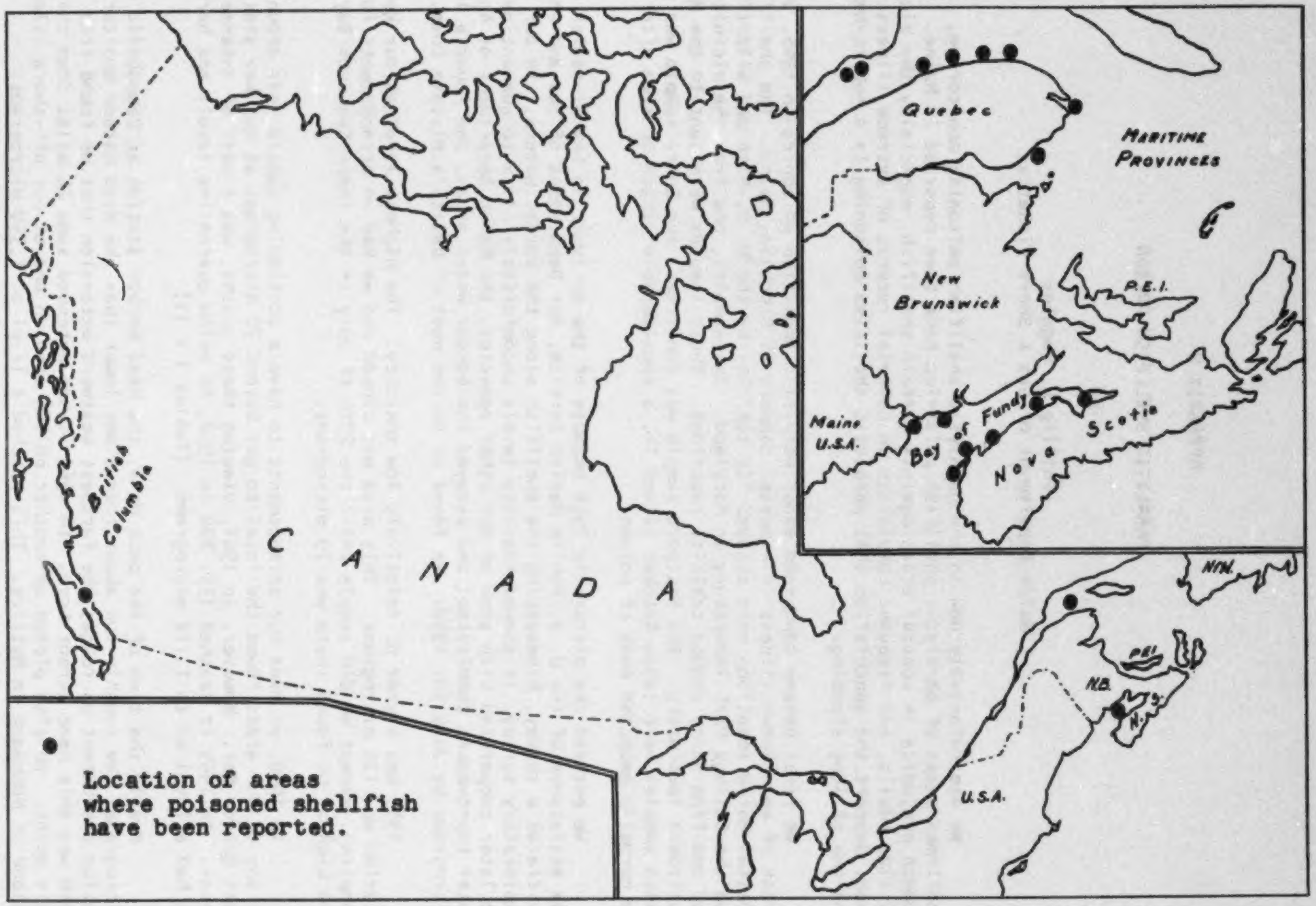
Location of areas where poisoned shellfish have been reported.
CANADA
British Columbia
Quebec
MARITIME PROVINCES
New Brunswick
P.E.I.
Maine U.S.A.
Bay of Fundy
Nova Scotia
NFN.
P.E.I.
N.B.
N.S.
U.S.A.

APPENDIX W

PARALYTIC SHELLFISH POISON

by

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Maine Department of Sea & Shore Fisheries

We are relatively new in the paralytic shellfish poisoning monitoring. So far, no confirmed cases of paralytic shellfish poisoning have been reported in Maine. Still, the common prejudice in coastal areas against certain shellfish, especially the blue mussel, Mytilus edulis, and frequent complaints in colonial records of extreme illness from eating them, support the speculation that paralytic shellfish poisoning is a public-health problem and one of long standing.

We first became concerned about paralytic shellfish poisoning in 1945, after an outbreak of an unknown illness in a mussel cannery at Brooklin, Maine. The shellfish from coastal Maine locations were assayed for toxicity by the U. S. Fish and Wildlife Service and the College Park laboratory in Maryland. Two samples, one from the vicinity of Eastport and one from Surry showed toxicity reactions. These samples were sent to the Massachusetts antitoxin laboratory. The Eastport sample was confirmed, the Surry sample was not. However, these samples were taken October 12 and 14; a considerable length of time after the would be normally expected peak of poison.

We entered the picture in 1958 because of the epidemic in New Brunswick of 1957. With the assistance of the U. S. Public Health Service, our Department of Sea and Shore Fisheries initiated a survey, bioassaying the shellfish along the border waters. In our first exploratory survey, it showed toxicity levels uncomfortably close to quarantine gram level. We later cooperated with some of our other agencies, the Maine Department of Agriculture and Water Improvement Commission, and assayed the border water areas, and found a level of 240 micrograms by July 10, 1958. We found no poison west of Bailey's Mistake Cove.

1959 was a year of relatively low toxicity. The highest level at our key monitoring station was 135 micrograms. This area was closed and we had no arrangements for continuing sampling, except we did sample until the 20th of July in the inner Cobscook Bay area and the highest we found there was 79 micrograms.

In 1960, we made our arrangements to have a continuing sample year around. We never in any of our areas found the level to get beyond 70 micrograms at our key station of West Quoddy Bar. However, in 1961, viewing these graphs, was a year of intense toxicity level. In 1959 it reached 135; 204 in 1958, 60 below quarantine level; and here in 1961 we had a level up to 1,112 micrograms. (Tables I & II)

Nearly the time of the peak here, the Head Harbor Station at Campobello was 5,500 micrograms. We roughly run about four times lower than the Head Harbor monitoring station. Notice the extent of range; the farthest westward extension that we found from 1958 through 1960 was this Head Harbor Cove. In 1961 it had extended some 25 miles down the coast to this point. We also picked up another poison area on one of our off-shore islands, the island of Monhegan in Mytilus. This reached a level of 1,550 micrograms.

Furthermore, on some of the islands that lie near Mount Desert Island, it showed less than 60 micrograms. However, we got poison readings in the 50's, 54 and 57 past Head Light. And the island of Matinicus, taken considerably after the peak, showed an indication of poison. There was poison in Mytilus, and westward of Southport Island, 57 at Head Harbor Beach, 62 in Mytilus, and into the very westward part of Maine, at Biddeford Pool, 57 micrograms. I understand that the Commonwealth of Massachusetts found 38 micrograms at Essex Beach.

The year of 1961, we expected there would be some reports of shellfish poisoning. We alerted our coastal health officers. We were advised of only one potentially dangerous incident. One of the local fisherman at the Head Harbor Beach area harvested some surf clams, Spisula solidissima, for his own use. In preparing them for freezing, he cut out the edible parts and put them in a frozen package and fed the visceral mass to the chickens and cat. The chickens died immediately; the cat which had just nibbled on the visceral mass became violently ill. About ten days afterwards the fellow thought he better send them over to us, and we assayed the rims and the foot of the Spisula and it showed a level of 162 micrograms.

Later, in picking up Spisula from that area, we tried to get some reading on where the poison was concentrated, and after the peak of intensity was over, we found in Spisula the whole clam, the entire meats showed 56, the rims and foot 38, and the visceral mass, 106 micrograms. The bar clams seem to hold the poison much longer than Mya and Mytilus. We held these same in our tanks at Bothbay Harbor for three weeks and it held the poison about level until the third week, and then it dropped down beyond the sensitivity of our test which is 32.

Incidental to our monitoring, we tried to get some evaluation of some of the other resident species in the area and the two things we have noted, Polynices heros concentrated the poison much stronger than Mytilus and Mya. On four samples of Polynices heros, when Mytilus was less than 38, Polynices was 80; in another case, when Mya was 62, Polynices was 120. The Rock Purple Thais, when Mytilus was less than 32, the Rock Purple was 34, with Zirfaea, the Great Pit clam, Mya was 56, Zirfaea contained 152 micrograms. Zirfaea is a mud borer and occupied a lower zone in the flats. Another thing we found is in Cancer borealis when Mytilus was 47, we found 56 micrograms in Cancer borealis. (Table III)

We have a monitoring scheme which is essentially the same type of scheme as conducted in Canada. We sample mostly at our cape monitoring station during the winter time from May 1 on until the first indication of poison every two weeks. When the poison is there, we sample weekly until October 1, or when the poison gets down into safe levels.

We have two designations for control. We have the provisionally open areas and the provisionally open areas are those which have contained poison beyond the 80 microgram level. The other area, we call it just probable area and that is any area where we may find the poison. So our provisional open area which we have considered north and east of the Bailey's Mistake Cove until 1960 is extended now down to the Little Kennebec River some 25 miles down the coast and also another one here at Monhegan Island. We have closed Monhegan Island and we since there is no commercial fishery there intend to keep it closed.

We were a little surprised that we didn't have any difficulty from the Monhegan Island area, because there is a group of summer people Germans, who have a common habit of going down and having mussel parties, and we thought certainly that we would get a case out of there.

It points out again that we do need more of the work, as Dr. Prakash has been doing. It is very difficult to relax with a monitoring system based on shellfish assays alone. We need a much broader base of knowledge to make our predictions more precise. We hope to use the Monhegan station as a Dew Line station. This held the poison about a week longer. Unfortunately we did not get the sampling there at the beginning of the rise of the poison. We got it near the peak and when it was on its way out. But it held the poison roughly a week longer than our most sensitive monitoring station and border areas.

TABLE I

MONITORING RESULTS ON PROVISIONALLY OPENED AREAS

1. West Quoddy Bar

Micrograms poison per 100 grams meat

1958 Mya		1959 Mya		1960 Mya		1961 Mya	
7/1	69	3/13	less than 62	1/20	less than 54	1/31	less than 38
7/10	204	3/25	less than 62	2/24	less than 54	2/28	40
7/24	104	4/15	60	5/8	less than 40	3/30	less than 38
7/29	160	5/12	63	5/20	47	4/28	46
8/5	56	5/26	less than 62	5/21	less than 40	5/19	37
8/13	less than 62	6/10	less than 62	5/24	48	6/2	less than 32
8/26	50	7/1	135	6/1	less than 40	6/14	less than 32
9/2	less than 62	7/8	62	6/7	41	6/29	110
9/9	58	11/16	less than 54	6/16	42	7/7	112
9/23	less than 62	12/8	less than 54	6/22	45	7/10	292
10/8	78	12/30	less than 54	6/29	48	7/19	602
10/21	less than 62			7/5	49	7/27	1112
11/5	80			7/13	less than 38	8/3	660
11/18	less than 62			7/19	63	8/10	58
12/1	less than 62			7/24	38	8/17	105
12/3	108			7/27	70	8/23	96
12/9	less than 62			7/30	55	8/31	54
12/17	less than 62			8/3	58	9/7	63
12/24	less than 62			8/7	56	9/14	41
12/30	less than 62			8/10	50	9/21	34
				8/16	44		
				8/23	40		
				8/31	less than 38		
				9/7	39		
				9/16	38		
				10/4	less than 38		
				10/11	less than 38		
				10/26	42		
				11/30	less than 38		
				12/28	47		

TABLE I, Con't.

2. Leighton Point

Micrograms poison per 100 grams meat

1959 Mya		1960 Mya		1961 Mya	
7/8	66	2/24	54	1/12	less than 38
7/15	68	5/8	less than 40	6/29	less than 50
		5/16	less than 40	7/7	58
		5/24	less than 40	7/10	158
		5/31	less than 40	7/19	296
		6/7	less than 40	7/27	497
		6/15	less than 40	8/3	302
		6/21	less than 40	8/10	35
		6/29	less than 40	8/23	43
		7/5	less than 38	9/1	32
		7/13	less than 38	9/7	31
		7/20	42	9/14	41
		7/26	less than 38	9/21	32
		8/3	38		
		8/10	less than 38		
		8/16	less than 38		
		8/24	less than 38		
		8/31	less than 38		
		9/7	less than 38		
		9/16	less than 38		
		10/4	less than 38		

3. Old Perry-Eastport Bridge

1958 Mya		1959 Mya		1960 Mya		1961 Mya	
7/10	69	3/9	less than 62	2/24	less than 54	1/12	less than 38
7/23	less than 62	3/23	less than 62	5/8	less than 40	6/29	less than 50
		4/14	less than 62	5/16	less than 40	7/11	162
		5/11	60	5/24	less than 40	7/19	161
		7/8	56	5/31	less than 40	7/27	440
				6/7	less than 40	8/3	276
				6/15	less than 40	8/10	30
				6/21	44	8/23	46
				6/29	less than 40	9/7	less than 32
				7/5	less than 38	9/14	less than 32
				7/13	less than 38	9/21	39
				7/20	41		
				7/24	less than 38		
				7/26	less than 38		
				8/3	less than 38		
				8/10	less than 38		
				8/16	less than 38		
				8/24	less than 38		
				8/31	less than 38		
				9/7	less than 38		
				9/16	less than 38		
				10/4	less than 38		

TABLE I, Con't.

4. Birch Point

Micrograms poison per 100 grams meat

1958 Mya		1959 Mya		1960 Mya	
7/22	less than 62	6/9	less than 62	6/15	less than 40
7/10	less than 55	6/30	less than 62	7/5	less than 39
		7/7	less than 62	7/13	less than 39
				7/20	less than 38
				7/26	less than 38
				8/3	less than 38
				8/11	less than 38
				8/16	less than 38
				8/24	less than 38
				8/31	less than 38
				9/8	less than 38
				9/16	less than 38

5. Mill Cove

1958 Mya		1960 Mya		1961 Mya	
7/10	less than 62	5/31	less than 40	7/27	81
7/29	less than 62	6/21	less than 40		
		7/13	less than 38		
		7/20	less than 38		
		7/26	less than 38		
		8/4	less than 38		
		8/11	less than 38		
		8/25	less than 38		
		9/8	less than 38		
		10/26	less than 38		

6. Frost Island

1958 Mya		1959 Mya		1960 Mya	
7/10	71	3/13	less than 62	1/25	less than 54
7/28	61	3/25	less than 62	7/13	less than 38
8/5	less than 62	4/15	less than 62		
8/14	less than 62	5/12	less than 62		
8/26	less than 62	5/26	less than 62		
9/9	less than 62	6/10	less than 62		
9/23	less than 62	7/1	59		
10/8	less than 62	7/22	79		
10/21	less than 62	11/16	less than 54		
11/5	less than 62	12/26	less than 54		

TABLE I, Con't.

7. Moose Island

1958 Mya		1959 Mya		1960 Mya		1961 Mya	
7/10	85	7/8	66	7/15	less than 40	7/11	169
7/22	77	7/15	58	8/24	less than 38		
8/6	less than 62	7/20	71				
8/14	less than 62						
8/26	less than 62						
9/9	less than 62						
9/23	less than 62						
10/8	less than 62						
10/21	less than 62						
11/5	less than 62						

8. Bailey's Mistake

1958 Mya		1959 Mya		1960 Mya		1961 Mya	
7/24	less than 62	7/7	63	6/15	less than 40	7/7	75
7/29	less than 62			7/5	less than 38	7/11	77
				7/13	less than 38	7/18	168
				7/20	less than 38	7/24	581
				7/24	less than 38	8/3	301
				7/30	45	8/10	42
				8/7	less than 38	8/24	less than 32
				8/10	less than 38	8/31	less than 32
				8/24	less than 38		
				9/7	less than 38		

9. Moose Cove

1961 Mya	
7/18	641
7/24	1108
8/7	627
8/24	60
9/5	90
9/15	39
9/19	42
9/22	43

TABLE I, Con't.

10. Little Machias Bay

1958 Mya		1959 Mya		1960 Mya		1961 Mya	
7/2	less than 62	7/8	less than 62	6/16	less than 40	7/7	less than 50
7/19	less than 62	7/13	less than 62			7/18	less than 44
7/29	less than 62	7/25	less than 62			7/24	170
						8/7	69
						8/15	less than 32
						8/29	less than 32
						9/5	less than 32
						9/8	less than 32
						9/19	less than 32

11. Holmes Bay, Randall Point Flats

1958 Mya		1959 Mya		1960 Mya		1961 Mya	
7/6	less than 62	7/7	less than 62	6/16	less than 40	7/18	less than 44
7/19	less than 62	7/24	less than 62			7/24	less than 44
						8/2	less than 54
						8/4	less than 32
						8/8	less than 44
						8/11	less than 32
						8/29	less than 32

12. Hplmes Bay, Township Rock

1958 Mya		1959 Mya		1960 Mya		1961 Mya	
7/2	less than 62	7/30	less than 62	-		7/18	less than 44
						7/24	less than 44
						7/26	less than 44
						8/2	less than 54
						8/4	less than 32
						8/8	less than 44
						8/29	less than 32

13. Holmes Bay, Ackley Beach

-	-	-	1961 Mya	
			7/18	less than 44
			7/26	less than 44
			8/2	less than 54
			8/4	less than 32
			8/8	less than 44
			8/11	less than 32

TABLE I, Con't.

14. Holmes Bay, Davis Cove

1958 Mya	1959 Mya	1960 Mya	1961 Mya	
-	-	-	8/2	less than 54
			8/4	less than 32
			8/31	less than 32
			9/18	less than 32

15. Holmes Bay, Little Bay

			1961 Mya	
-	-	-		
			7/26	less than 44
			8/4	less than 32
			8/8	less than 32

16. Holmes Bay, Larrabee Cove

			1961 Mya	
-	-	-		
			7/26	less than 44
			8/4	less than 32
			8/8	less than 44

17. Holmes Bay, Salt Island

			1961 Mya	
-	-	-		
			7/28	less than 44

18. Holmes Bay, Hog Island

			1961 Mya	
-	-	-		
			7/28	less than 54

TABLE I, Con't.

19. Starboard Island Bar

1958 Mya	1959 Mya	1960 Mya		1961 Mya	
-	-	5/19	less than 40	7/29	396
				8/2	174
				8/11	46
				8/18	70
				8/24	44
				9/5	less than 32
				9/8	35
				9/12	less than 32
				9/15	less than 32
				9/19	less than 32

20. Monhegan Island

			1961 Mya	
-	-	-	7/30	1547
			8/9	785
			8/15	386
			8/31	47
			9/7	less than 32
			9/18	less than 32

TABLE II

MONITORING RESULTS ON PROBABLE POISON AREAS

Micrograms Poison per 100 Grams Meat

1959				1960				1961			
Date	Location	Species	ug/100gms.	Date	Location	Species	ug/100gm.	Date	Location	Species	ug/100gms.
7/25	Hancock Point	Mytilus	less than 62	6/14	Roque Bluffs	Mytilus	less than 40	4/20	Roque Bluffs	Mya	less than 38
7/25	Gouldsboro Bay, Corea	Mya	less than 62	6/14	" "	Mya	less than 40	7/18	" "	"	less than 44
7/25	Roque Bluffs	"	less than 62	6/14	Beals Is.	"	less than 40	7/26	" "	"	less than 44
7/29	Beals Is.	"	less than 62	7/6	Newbury Neck, Surry	Mytilus	less than 38	7/27	" "	Mytilus	less than 44
7/29	Ripley Neck	Mytilus	less than 62	7/12	" " "	"	less than 38	7/28	" "	"	less than 44
				7/21	" " "	"	less than 38	7/28	" "	Mya	less than 44
				7/28	" " "	"	less than 38	8/2	" "	"	less than 44
				8/4	" " "	"	less than 38	7/28	Roque Is.	"	76
				8/25	" " "	"	less than 38	7/18	Beals Is.	"	less than 44
				8/4	" " "	"	less than 38	7/26	" "	"	less than 44
				8/25	" " "	"	less than 38	7/15	Jonesboro Chandler Bay	"	less than 44
				8/4	Morgan's Bay	"	less than 38	7/26	"	"	less than 44
				8/2	Hancock	"	less than 38	7/26	Great Wass Island	"	less than 44
				7/21	Deer Isle, Sunshine	"	less than 38	8/2	Jonesport Great Bar	"	less than 44
				6/14	Blue Hill Harbor	Mya	less than 38	8/2	Jonesport Indian Riv.	"	less than 44
				7/27	Kennebec River	"	less than 38	8/1	Addison-Cape Split	"	less than 44
								8/3	Mt. Desert Is., SW Hbr.	"	49
								8/9	Mt. Desert Bass Hbr. Head	Mytilus	54
								8/16	Mt. Desert NE Hbr.	"	less than 32
								8/16	Mt. Desert Seal Cove	"	less than 32

TABLE II, Con't.

1961

Date	Location	Species	ug/100gms
8/8	Swans Is.	Mytilus	less than 44
8/14	Isle au Haut	"	less than 32
8/3	Stonington Sunshine	"	less than 54
8/3	" Crockett Cove	"	less than 54
8/3	" Oceanville Road	Mya	less than 54
8/3	" Webb Cove	"	less than 54
8/1	" Duryea Lobster Pound	Mytilus	less than 54
8/3	Dear Is. Little Deer Isle	Mya	less than 54
8/16	Sedgwick	Mya	less than 32
8/16	"	Mytilus	less than 32
8/3	Castine Holbrook Is.	Mya	less than 54
8/9	Newbury Neck Surry	Mytilus	less than 54
7/27	Lincolnville Ducktrap	Mytilus	less than 44
9/17	Matinicus Island	Mytilus	less than 32
8/1	Southport Cape Newagen	"	57
8/1	Southport Huskins Lobster Pound	"	less than 54
8/10	Sagadahoc Bay	Mya	less than 44
8/9	Kennebec R. Popham B.	"	less than 44
8/9	" " "	Mytilus	less than 44
8/2	Phippsburg Small Pt.	"	62
8/9	" " "	Mya	less than 44
8/29	" " "	"	less than 32
8/2	Casco Bay	"	less than 54
8/2	" Bailey Island	Mytilus	less than 54
8/9	Casco Bay Grassy Ledge	"	less than 44
8/9	" Duck Cove	"	less than 44
8/9	" Mere Point	"	less than 44
8/2	Biddeford Pool	"	59
8/19	" "	"	less than 32

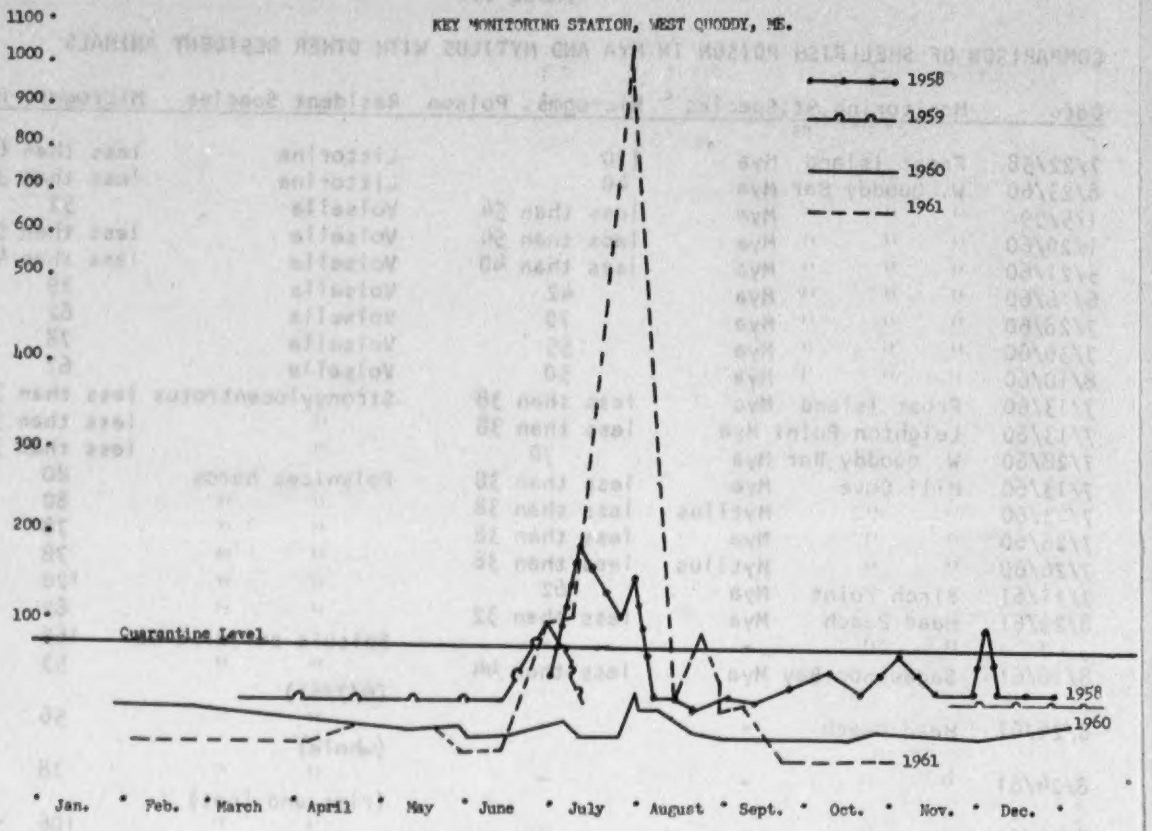
TABLE III

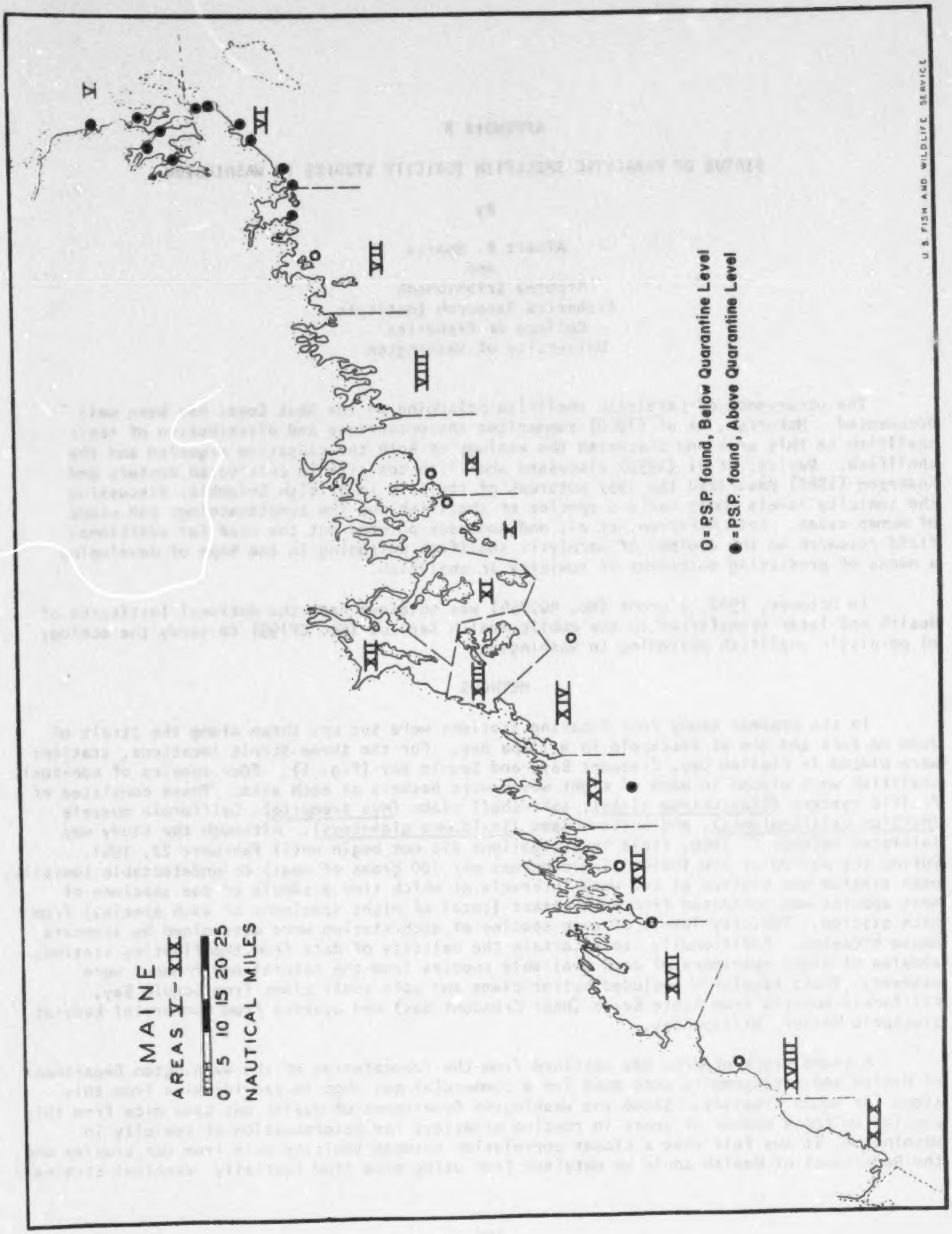
COMPARISON OF SHELLFISH POISON IN MYA AND MYTILUS WITH OTHER RESIDENT ANIMALS

Date	Monitoring St.	Species	Microgms. Poison	Resident Species	Microgms. Poison
7/22/58	Frost Island	Mya	110	Littorina	less than 62
8/23/60	W. Quoddy Bar	Mya	40	Littorina	less than 38
1/5/59	" " "	Mya	less than 54	Volsella	52
1/20/60	" " "	Mya	less than 54	Volsella	less than 54
5/21/60	" " "	Mya	less than 40	Volsella	less than 40
6/16/60	" " "	Mya	42	Volsella	39
7/28/60	" " "	Mya	70	Volsella	62
7/30/60	" " "	Mya	55	Volsella	78
8/10/60	" " "	Mya	50	Volsella	67
7/13/60	Frost Island	Mya	less than 38	Strongylocentrotus	less than 38
7/13/60	Leighton Point	Mya	less than 38	"	less than 38
7/28/60	W. Quoddy Bar	Mya	70	"	less than 38
7/13/60	Mill Cove	Mya	less than 38	Polynices heros	80
7/13/60	" "	Mytilus	less than 38	" "	80
7/26/60	" "	Mya	less than 38	" "	78
7/26/60	" "	Mytilus	less than 38	" "	78
7/11/61	Birch Point	Mya	62	" "	120
8/29/61	Head Beach	Mya	less than 32	" "	62
?	" "	-	-	Spisula solidissima	162
8/10/61	Sagadahoc Bay	Mya	less than 44	" " (8/7/61)	53
8/24/61	Head Beach	-	-	" " (whole)	56
8/24/61	" "	-	-	" " (rims and foot)	38
8/24/61	" "	-	-	" " (visceral mass)	106
8/29/61	" "	Mya	less than 32	Spisula solidissima	55
8/29/61	" "	-	-	" " (rims and foot)	less than 32
8/29/61	" "	-	-	" " (Visceral mass)	51
9/5/61	" "	-	-	" " (held in tank at Boothbay Harbor)	51
9/28/61	" "	-	-	Spisula solidissima (held in tank at Boothbay Harbor)	less than 32
9/28/61	" "	-	-	Spisula solidissima	59
8/31/61	Monhegan	Mytilus	47	Cancer borealis	56
8/19/61	Monhegan	Mytilus	less than 32	Thais	34
8/7/60	W. Quoddy Bar	Mya	56	Zirfaea	152
7/28/61	Surry	Mytilus	less than 38	Asteris	less than 38

POISON INTENSITY

KEY MONITORING STATION, WEST QUODDY, ME.




MAINE
AREAS V – XIX
0 5 10 15 20 25
NAUTICAL MILES
O = P.S.P. found, Below Quarantine Level
● = P.S.P. found, Above Quarantine Level
U.S. FISH AND WILDLIFE SERVICE
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
XVII
XVIII
XIX

APPENDIX X

STATUS OF PARALYTIC SHELLFISH TOXICITY STUDIES IN WASHINGTON

By

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The occurrence of paralytic shellfish poisoning on the West Coast has been well documented. McFarren, et al (1960) summarized the occurrence and distribution of toxic shellfish in this area and discussed the ecology of both the causative organism and the shellfish. Davies, et al (1958) discussed shellfish toxicity in cultivated oysters and Anderson (1960) described the 1957 outbreak of toxicity in British Columbia, discussing the toxicity levels among various species of shellfish and the symptomatology and scope of human cases. Both McFarren, et al, and Anderson pointed out the need for additional field research on the ecology of paralytic shellfish poisoning in the hope of developing a means of predicting outbreaks of toxicity in shellfish.

In October, 1960, a grant (No. RG7754) was obtained from the National Institutes of Health and later transferred to the Public Health Service (No. EF199) to study the ecology of paralytic shellfish poisoning in Washington.

METHODS

In the present study four floating stations were set up, three along the Strait of Juan de Fuca and one at Stackpole in Willapa Bay. For the three Strait locations, stations were placed in Clallam Bay, Crescent Bay, and Sequim Bay (Fig. 1). Four species of non-toxic shellfish were placed in each of eight woven-wire baskets at each site. These consisted of Pacific oysters (Crassostrea gigas), soft-shell clams (Mya arenaria), California mussels (Mytilus californianus), and butter clams (Saxidomus giganteus). Although the study was initiated October 1, 1960, field investigations did not begin until February 22, 1961. During the period of low (below 35 micrograms per 100 grams of meat) or undetectable toxicity, each station was visited at two week intervals at which time a sample of one specimen of each species was collected from each basket (total of eight specimens of each species) from each station. Toxicity levels of each species at each station were determined by standard mouse bioassay. Additionally, to ascertain the validity of data from the floating station, samples of eight specimens of each available species from the natural environment were assayed. These regularly included butter clams and soft shell clams from Sequim Bay, California mussels from Agate Beach (near Crescent Bay) and oysters from commercial beds at Stackpole Harbor, Willapa Bay.

A brood stock of mice was obtained from the laboratories of the Washington Department of Health and arrangements were made for a commercial pet shop to provide mice from this stock for mouse bioassay. Since the Washington Department of Health has used mice from this population for a number of years in routine bioassays for determination of toxicity in Washington, it was felt that a closer correlation between toxicity data from our studies and the Department of Health could be obtained from using mice from initially identical strains.

A supply of purified toxin was obtained from Dr. Edward J. Schantz and using the method outlined by Schantz, et al., (1958) determination of the correction factor (CF value) for the mice used in this study was made. (see Table 1.)

Plankton samples, consisting of one liter grab sample, were taken at each visit. These were immediately fixed by adding neutralized formalin to give a 5 per cent solution, were allowed to settle for one week and the dinoflagellates isolated and counted in a Palmer nannoplankton counting cell.

Recording thermographs are maintained at each station so that a constant record of water temperature is available. Dissolved oxygen, salinity, pH, nitrates, phosphates and silicates are determined from water samples from each site at each station check. Sunshine records are obtained from the weather stations at Tatoosh Island, Washington, and Vancouver Island, British Columbia.

From the above data it is hoped that the following information can be obtained; under identical field conditions, the species of shellfish that first attains high levels of toxicity; the species that becomes the most toxic; the retention rates of the four species; the geographic and seasonal pattern of toxicity; and the planktonic and environmental features associated with developing toxicities.

RESULTS

During the first season of investigation, some extremely interesting patterns of toxicity have developed. Because of the frequent sampling during the summer period of high toxicity levels, the processing of hydrographic and plankton samples has yet to be completed.

Toxicities in Willapa Bay:

The site selected for a station in Willapa Bay was Stackpole Harbor, the location in which toxic oysters were first found by Sommer in 1941 (McFarren, et al., 1960). Toxicities to date at the Willapa station have never reached levels higher than 35 micrograms (ug) per 100 grams of meat (Table 2). From the latter part of June to the middle of July, a low peak developed in the California mussel, but no detectable toxin has occurred in mussels since that time except in two samples (less than 27 ug) in the early part of October. Low levels of toxicity have occurred in a single instance in oysters and twice in butter clams from the floats, but detectable levels have never been found in oysters from the commercial beds.

Toxicities in the Strait of Juan de Fuca:

Toxicity levels in all species at all stations are presented in Table 2.

Levels of toxicity remained low in the Strait of Juan de Fuca stations from late February until the middle of June, at which time the toxicity in California mussels in the Clallam Bay station become elevated from 32 ug on June 2, to 400 ug on June 14 (Table 2), while toxicity levels in mussels in other stations remained below 50 ug. Two weeks later levels reached 161 ug in mussels at the Crescent Bay station (up from 50 ug the week before), while the level in mussels in the Sequim Bay float was still below 50 ug. However, on July 28, the toxicity in Sequim Bay rose suddenly from 39 ug the week before to 500 ug. These data seem to indicate that toxicities developed first in the most seaward station and moved in a progressive manner up the Strait (see Fig. 2). With very few exceptions, the other species in the floats follwed the same pattern, though lower peaks were attained, as the mussels. The same approximate situation was also shown in samples from the natural beds, with the exception of mussels from Agate Beach (adjacent to Crescent Bay) which did not attain levels of 50 ug when Crescent Bay mussels reached almost 165 ug in late June.

The data indicate that a second peak of high toxicity levels occurred throughout the Strait of Juan de Fuca, but with progressiveness of pattern not quite so marked as the first peak (Fig. 2). The high toxicity levels of mid-June in Clallam Bay mussels dropped off in late Juna and early July, but became elevated to approximately 500 ug in late July and reaching a peak of 760 ug on August 11, with a small peak of 350 ug in the interval during the middle of July. This small peak was probably reflected by the sudden elevation from 90 ug to 350 ug in Crescent Bay on July 28. If the third peak (July 13 in Clallam Bay) hit Crescent Bay, it was apparently masked by the preceding peak just mentioned. More likely, the major peak which developed July 13 in Clallam Bay was largely responsible for the continued elevation above 150 ug at Crescent Bay which lasted until August 23. The first peak of high levels in mussels in Sequim Bay, which began July 28, gradually dropped off to a low of 50 ug by September 9, but then became elevated to 270 ug by September 25. This, one can surmise, resulted from the July 13 peak in Clallam Bay again occurring several weeks after the peaking in Clallam Bay and further indicating the progressive nature of developing high levels. Although the data indicated a progression of toxicity from the outer coast inland, the alternative possibility exists that conditions became suitable for the development of toxicity only in the Clallam Bay region in June, simultaneously in all three areas in August and only in Sequim Bay in September.

Comparison of Toxicities Between Species in the Same Location:

Due to the difficulty of maintaining butter clams and soft shell clams on the floats, our data are not complete on the uptake and retention among the species. The problem of maintaining adequate populations of soft shell clams is apparently solved, but further work is necessary in the case of the butter clams. Of considerable value in this regard are the data on butter clams and soft shell clams from the natural beds in Sequim Bay.

We find that in all stations the California mussel attained high toxicity levels much more quickly than other species, although the Pacific oyster occasionally showed a rise prior to or at the same time as the mussel (See Table 1). In addition to becoming highly toxic more rapidly than the other species studied, the mussel, with the one exception of butter clams from the natural bed in Sequim Bay in September, attained higher levels of toxicity than any species in the study.

Following the mussel in degree of toxicity, we find the butter clam reaching the next highest levels, followed by the soft shell clam and finally the oyster.

The oyster was found to lose its toxicity more rapidly than the other species in most instances, although the soft shell clam possibly declines in level at about the same rate. Somewhat surprisingly, the mussel, which is generally considered to become toxic rapidly, but to also decline rapidly, appeared to decline in toxicity more slowly than any species except the butter clam. This phenomenon is probably related to the higher toxicities attained and resulting in a longer period necessary for loss of toxicity. As previous workers have noted, the butter clam acquires high toxicity levels more slowly than the other species, but retains the high levels for a longer period.

Analysis of the plankton samples is not yet complete, but Gonyaulax sp., probably G. catenella, has been collected at each strait station, with the greatest numbers apparently occurring prior to or during the peaks of toxicity.

Hydrographic Data:

The hydrography of the Strait of Juan de Fuca and Puget Sound have been extensively studies since 1932, with the exception of the war years of 1942-1947 (University of Washington, 1954). In general, fresh water runoff and precipitation exceeds evaporation in Puget Sound and the Strait of Juan de Fuca with excess fresh water being carried seaward on the surface and with a net influx of salt water coming in from the Pacific Ocean at depth. This more saline water is diluted somewhat by mixing in shoals at the eastern end of the strait and in the connecting channels to the Strait of Georgia and Puget Sound. Because the Pacific Ocean is a pronounced heat sink, the coldest subsurface water of the year penetrates the Strait of Juan de Fuca during the summer. A portion of this water mixes at the sills with the warmer, more dilute surface water flowing seaward at the surface and the mixed flows inland at or near the bottom. During winter the temperatures of the deeper Strait water is at its yearly maximum while the upper water in the Strait is cooled locally by the atmosphere and by mixing with the cold water flowing into the Strait from the Strait of Georgia.

Upwelling of ocean water apparently occurs off the coast of Washington in the summer, analagous in some respects to the situation off California. It is this upwelled water that is brought into the Strait of Juan de Fuca at depth and it is then brought to the surface in the Strait by vertical turbulence accompanying the strong tidal currents. This upwelled water is extremely rich in nutrients, particularly in phosphates, and may well be responsible for the sudden elevation of toxicity levels through blooms of dinoflagellates developing from the increased nutrients. If this is the case, we would expect to find the water mass just prior to or during the increased toxicities identifiable by increased salinity, lowered temperature, and higher phosphate content compared to the water mass prior to the outbreak of high toxicity level.

A portion of the hydrographic data obtained in the study is presented in Table 3. The water temperatures included are those obtained by thermometer at each station check rather than the temperature data from the recording thermographs which are incomplete because of delays in obtaining the instruments and breakdowns during operation at two of the four stations. Daily maxima, minima and 5-day average temperatures from the recording thermographs have been plotted for Sequim Bay and Crescent Bay stations, but have not been presented. Also omitted from the table are the dissolved oxygen and pH values obtained at each station check.

No obvious correlation between evironmental conditions and toxicity is apparent, although it appears that peaks in toxicity levels are preceded by a rise in water temperatures. This may be caused by an increase in feeding rates of the shellfish with the rise in temperature or an increase in multiplication rates of the dinoflagellates or by both. When the plankton counts are completed, the role of the plankton in this phenomenon will be more apparent.

Statistical analysis of the available environmental data will be conducted before toxicity levels rise in the coming spring. Multiple correlation analysis is being considered in an attempt to determine what set of environmental phenomena are related to the occurrence of toxicity in the shellfish.

ACKNOWLEDGEMENTS

A large number of individuals and organizations have been of great help in the planning and initiation of this investigation. Appreciation is expressed particularly to Mr. John Girard of the Washington Department of Health for valuable suggestions in the selection of station sites, for making available an original breeding population of mice, and for continued interest and valuable suggestions during the progress of the investigation. Mr. Clyde Sayce of the Washington Department of Fisheries provided valuable assistance in setting up and running the station in Willapa Bay. Land owners and resort operators at each site on the Strait of Juan de Fuca and Willapa Bay have been most helpful in granting access to the stations and in aiding the work in many ways. Valuable technical assistance in the field and laboratory have been provided by Miss Daphne Morris and Mr. Kenneth K. Chew of the Fisheries Research Institute of the College of Fisheries. Salinity, nitrate, and phosphate determinations have been run by the Department of Oceanography, University of Washington.

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University of Washington Department of Oceanography. 1954. Puget Sound and Approaches. A literature survey. Vol. 3:175 pp.

Figure 1

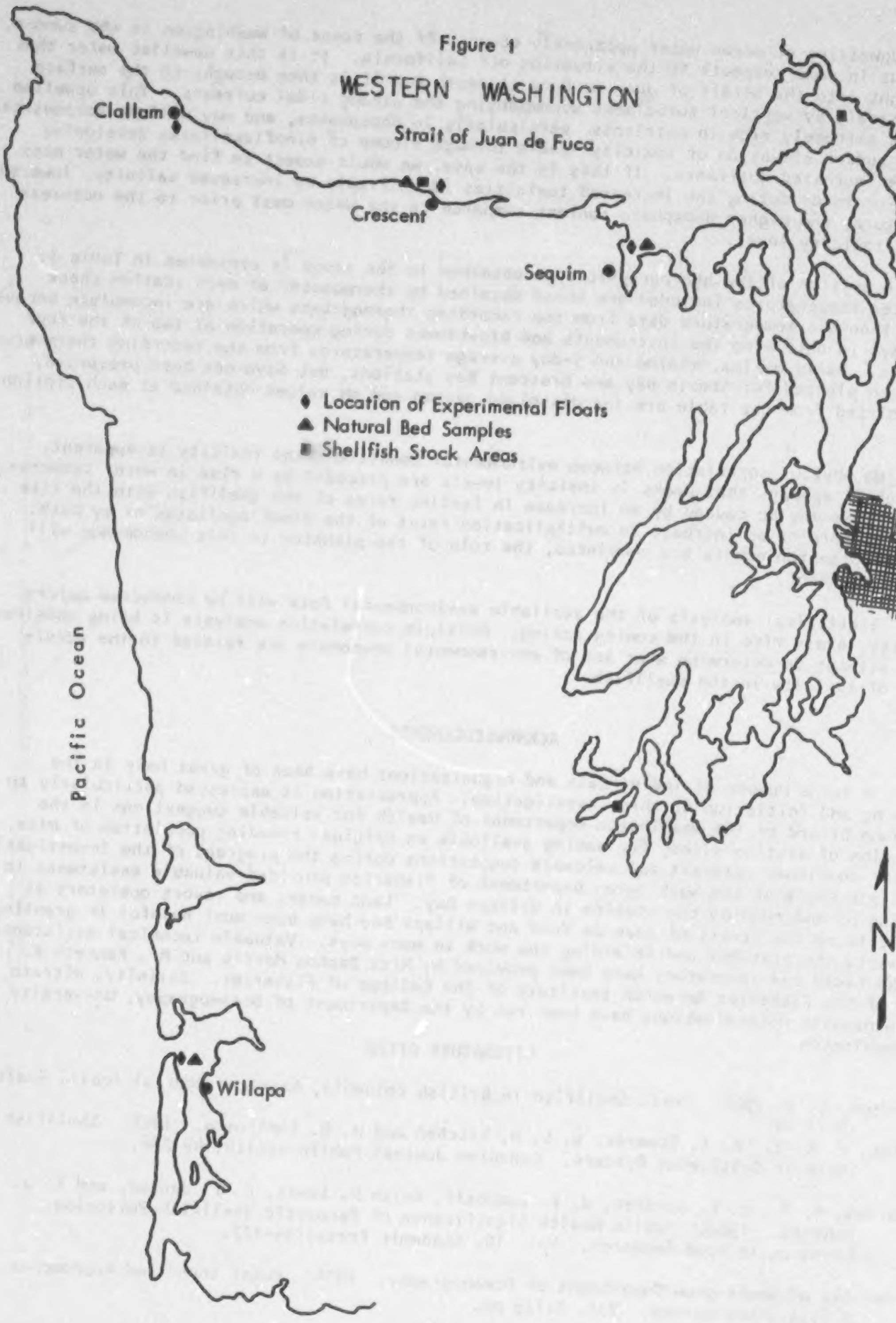
WESTERN WASHINGTON
Clallam
Strait of Juan de Fuca
Crescent
Sequim
Seattle
Pacific Ocean
Willapa
N
♦ Location of Experimental Floats
▲ Natural Bed Samples
■ Shellfish Stock Areas

Figure I

Toxicity levels in California mussel in Strait of Juan de Fuca stations, 1961

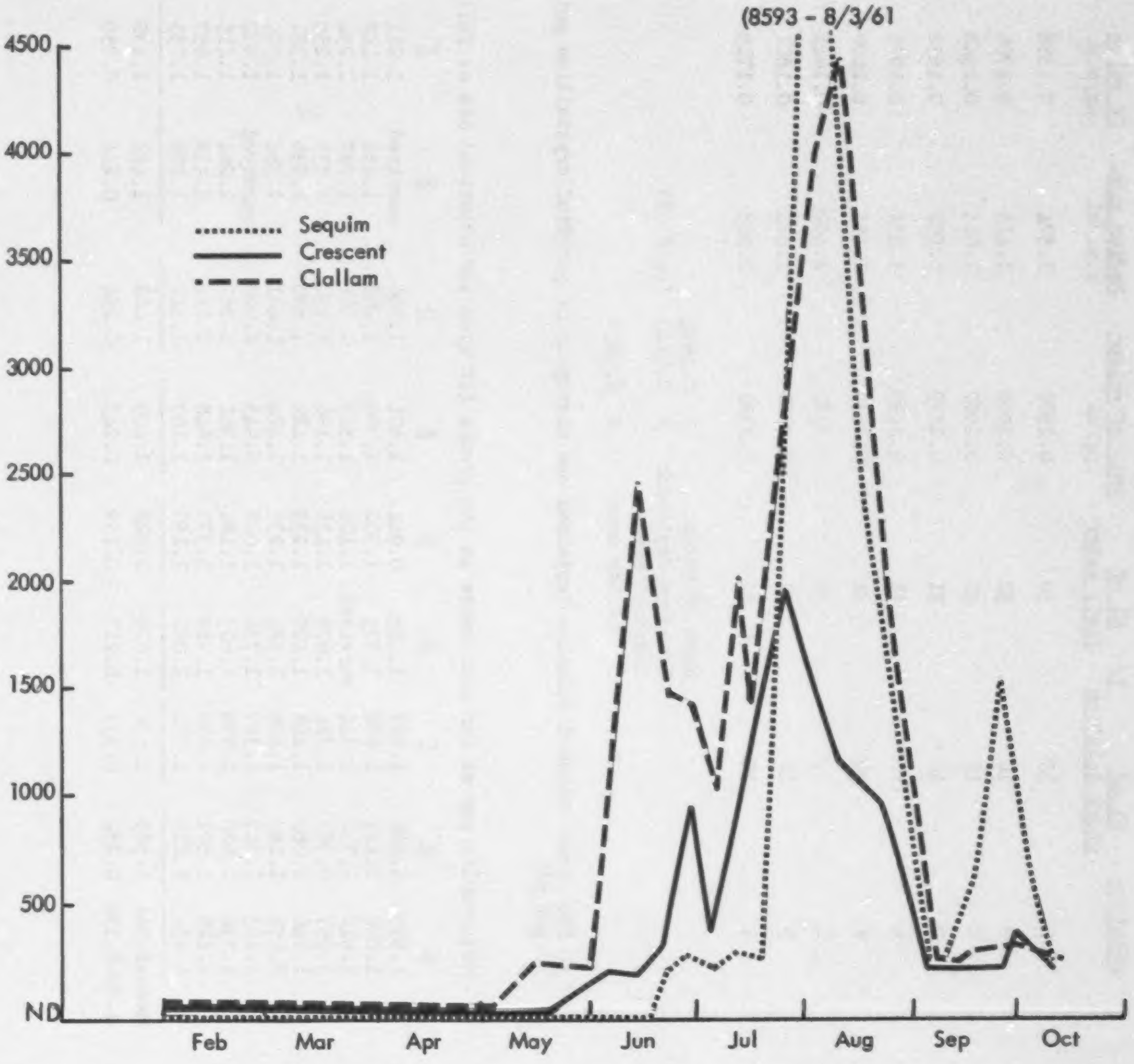

Table 1

DETERMINATION OF "CORRECTION FACTOR" (CF VALUE) FOR MOUSE-BIOASSAY

Dilution	ml of stock solution 1/	ml of dist. water	Amt. of poison µg/ml	Median m.u. m.u./ml	CF value µg/m.u.
A	10	30	0.2500	1.574	0.1588
B	10	30	0.2500	1.415	0.1766
C	10	30	0.2500	1.615	0.1548
D	10	31	0.2439	1.592	0.1532
E	10	31	0.2439	1.234	0.1975
F	10	31	0.2439	1.455	0.1676
G	10	32	0.2381	1.450	0.1641
H	10	32	0.2381	1.321	0.1801
I	10	32	0.2381	1.388	0.1715

Mean CF value = 0.1694
Standard deviation = 0.0140 (or 8.3%)
Standard error of the mean = 0.0047

1/ The stock standard solution contained one microgram of purified crystalline poison per ml.

Mouse-units per ml for each mouse in the groups (10 mice per dilution) are as follows:

	A	B	C	D	E	F	G	H	I
	1.935	1.661	1.985	1.455	0.944	1.601	1.290	survived	1.211
	1.000	1.484	1.678	1.375	1.302	1.709	1.208	1.451	1.149
	1.612	1.732	1.744	survived	1.401	1.549	1.564	1.287	1.234
	1.057	0.983	1.324	1.678	1.431	1.196	1.490	1.537	1.255
	1.746	1.080	1.248	1.409	1.233	1.487	1.180	1.936	1.381
	2.056	1.183	1.448	1.581	1.236	1.424	1.461	1.354	1.394
	1.623	1.347	1.329	1.731	1.027	0.945	2.009	survived	1.930
	1.536	1.605	1.579	1.603	1.184	1.361	1.262	1.264	1.415
	1.426	1.501	1.650	1.749	1.377	1.648	1.714	1.422	1.657
	1.454	1.252	1.751	2.080	1.150	1.167	1.440	1.109	1.753
Mean=	1.544	1.383	1.574	1.629	1.228	1.409	1.462	1.421	1.438
S.D.=	0.336	0.254	0.233	0.217	0.159	0.243	0.254	0.244	0.259

Table 2

Toxicity Level During February to December, 1961 in micrograms (ug) per 100 grams of meat

	WILLAPA BAY					CLALLAM BAY				CRESCENT BAY					SEQUIM BAY					
	Experimental Float				Nat. Beds	Experimental Float				Experimental Float				Agate Mussel	Experimental Float				Nat. Beds	
Date	Mussel	Oyster	Butter Clam	Softshell Clam	Oyster	Mussel	Oyster	Butter Clam	Softshell Clam	Mussel	Oyster	Butter Clam	Softshell Clam	Mussel	Mussel	Oyster	Butter Clam	Softshell Clam	Butter Clam	Softshell Clam
2-22		ND	ND				ND	ND			ND	ND		ND		ND	ND			
3-12	ND					ND				ND					ND					
4-16	ND	ND	ND			ND	ND	ND		ND	ND	ND		ND	28	ND	ND	ND		
5-6	31	ND	ND			ND	ND	ND		ND	ND	ND		ND	ND	ND	ND	ND		
5-17 / 5-22	ND	ND	ND			41	ND	ND		ND	ND	ND		ND	ND	ND	ND	ND		
6-2 / 6-8	ND	ND	ND		ND	32	ND	ND		30	ND	ND		ND	ND	ND	ND	ND	39	
6-14	No samples				ND	424	57	39	ND	<27	*	*		ND	ND	*	*	ND	37	
6-22 / 6-23	29	ND	ND		ND	253	42	<27	<27	50	37	42		34	34	<27	<27	<27	35	30
6-29	35	<27	ND		ND	246	39	79	53	161	32			35	47	32	36	31	41	ND
7-6	31	ND	ND		ND	192	43	89	35	113	29			60	35	ND	ND	ND	51	ND
7-13	29	ND	ND	ND	ND	358	29	69	41	72	ND		ND**	77	51	ND	35	30**	42	30
7-19 / 7-20	<27	ND	ND	ND	ND	238	52	38	50	94	29		ND	47	39	32		48	36	37
7-26 / 7-28	ND	ND	ND	ND	ND	504	70		ND***	353	40		46	444	534	303	227	222	225	62
8-3	ND	ND	ND	ND	ND	670	177		ND	309	43		ND	329	1456	379	321	560	867	207
8-9 / 8-11	ND	ND	ND	ND	ND	768	209		<27	270	55		42	334	809	74		227	568	
8-17	ND	ND	ND	ND	ND	601	42		29	220	33		46	228	420	38			251	
8-23	ND	ND	ND	ND	ND	320	36		35	167	35		38	134	307	30		42	290	29
9-2 / 9-5	ND	ND	ND	ND	ND	53	ND		ND	35	ND		ND	40	42	33		36	156	32
9-9 / 9-13	ND	ND	ND	ND	ND	41	ND		32	37	ND		ND	36	49	29		39	112	31
9-18	ND	ND	<27	ND	ND	52	<27		35	38	ND		30	36	123	113		125	284	36
9-25 / 9-26	ND	ND	ND	ND	ND	58	42		46	39	31		34	42	270	46		82	188	40
10-2	<27	ND	ND	ND	ND	54	33		39	66	32		39	56	135	36		37	196	38
10-10	<27	ND	<27	<27	ND	48	30		34	45	ND		32	40	51	<27		47	167	33
10-18	ND	ND			ND	37	ND		34	35	ND		31	34	32	ND			105	33
10-27	ND	ND			ND	32	ND		ND	30	ND		ND	31	34	ND		34	39	
11-7	ND	ND			ND	32	ND		ND	ND	ND		ND	ND	31	ND		34	36	ND
11-20	ND	ND			ND	32	33		ND	34	ND		ND	30	35	ND			75	
12-8	ND	ND			ND	32	ND		32	30	ND		<27	30	<27	ND		31	157	no sample
12-21	ND	ND			ND	-	-		-	29	ND		ND	34	34	ND		-	68	no sample

* Values are omitted due to an error in the laboratory procedure.

** A new population was introduced into the study 7-10-61; the toxicity values hence are those of the new population

*** A new population was introduced into the study 7-25-61.

Table 3 A Summary of Hydrographic Data At The Four Experimental Sites and The Average Daily Total Sunshine At Victoria and Tatoosh Island During 1961

Date	Temperature - °F				Nitrates - ug. at/L				Phosphates - µg. at/L				Salinity - o/co				Average Daily 1/ Total Sunshine-Hr	
	SQ*	CR*	CL*	WP*	SQ*	CR*	CL*	WP*	SQ*	CR*	CL*	WP*	SQ*	CR*	CL*	WP*	Victoria	Tatoosh
2/19-25	46.8	46.0	45.8	47.0	-	-	-	-	-	-	-	-				18.13	1.6	1.4
3/26-4																	3.9	5.7
3/5-11																	2.6	2.9
3/12-18	48.0	46.8	47.0	48.1	-	-	-	-	-	-	-	-				17.00	4.3	2.2
3/19-25																	4.8	3.3
4/26-1																	4.8	3.4
4/2-8																	7.4	8.7
4/9-15	-	-	-	54.0	7.7	16.3		2.1	3.40	2.04		1.12	30.21	31.58		26.18	3.7	4.1
4/16-22							14.2				1.95				31.04		4.7	3.8
4/23-29																	3.9	2.7
5/30-6	50.0	52.0	51.0	56.0	0	5.5	7.6	0	1.22	1.22	1.35	0.73	30.17	30.08	-	21.44	3.9	5.3
5/7-13																	4.0	2.4
5/14-20	54.5	51.3	52.0	64.0	0	16.6	9.1	0	0.49	0.49	1.30	0.47	30.14	31.36	-	24.04	13.3	4.8
5/21-27																	6.1	5.2
6/28-3	59.0	50.9	55.4	62.6	0.3	14.0	15.0	0.7	1.03	2.09	1.81	0.95	30.21	31.53	28.21	24.02	12.8	6.2
6/4-10																	5.3	0.9
6/11-17	58.1	54.5	59.9	66.0	0	7.4	9.9	-	0.59	1.80	1.30	-	30.32	31.46	28.59	20.52	14.0	10.2
6/18-24	59.9	56.3	54.5	65.3	2.7	3.4	8.0		1.11	1.26	2.43	2.04	29.79	29.54	30.64	22.65	14.3	7.8
7/25-1	59.9	54.5	54.5	62.4	6.6	2.6	3.5	0.5	0.83	1.35	0.92	0.80	29.29	30.77	-	24.20	10.2	
7/2-8	58.6	50.9	51.0	65.3	6.6	21.7	19.2		1.01	2.19	2.05	1.77	30.08	31.78	32.21	24.58	8.7	
7/9-15	64.4	52.7	53.6	68.0	0	20.4	14.9		0.88	2.29	2.15	1.17	30.41		31.20	27.23	13.1	
7/16-22	61.5	55.2	53.5	67.5	2.5	4.0	3.2	3.1	1.37	2.13	2.14	1.03	30.77	31.31	31.06	26.83	13.2	
7/23-29	63.5	59.3	52.0	63.0	0.7	0	12.0	1.1	1.60	0.88	5.64	-	30.52	31.49	31.76	29.56	11.5	
8/30-5	62.6	55.0	55.0	66.4	-	7.7	12.0	4.3	-	2.04	2.34	2.02	30.77	32.01		-	12.6	
8/6-12	62.6	54.3	52.0	62.0	2.4	17.7	22.8	4.3	2.08	2.06	1.39	1.63	30.79	31.62	32.25	29.99	13.6	8.8
8/13-19	60.8	56.3	52.7	62.7	4.0	17.5	8.5	2.5	1.56	1.97	1.39	1.37	30.79	31.40	31.36	29.29	7.5	1.6
8/20-26	57.2	55.4	55.4	62.0	9.2	7.9	2.5	7.0	1.80	1.27	1.24	1.66	30.84	31.13	31.11		10.8	8.8
9/27-2	55.4	53.2	52.1	64.4	12.4	5.9	26.8	5.9	2.04	3.03	2.67	1.23		32.29		29.72	9.4	
9/3-9	57.2			62.2	3.8			22.5	1.28			2.51				30.19	9.2	
9/10-16		50.9	49.1			1.7	27.5			1.25	2.52				31.67		8.5	
9/17-23	55.4	52.1	53.6	62.2	12.5	23.9	25.5	2.2	2.04	2.34	2.24	1.70	31.31	31.62	31.33	29.87	8.8	
9/24-30	55.4	50.9	50.0	56.3	18.7	22.5	12.0	9.7	1.61	2.20	2.20	1.89	31.65	32.88	32.52	32.50	4.1	
10/1-7	53.4	48.2	49.3	61.2	9.7	5.1	17.3	10.8	1.22	2.31	2.67	1.93	31.47	33.24	32.92	29.96	7.5	7.5
10/8-14	51.8	48.0			4.3	7.7	6.7	7.7	1.27	2.38	2.41	1.75	30.84	30.50		31.74	3.5	4.8
10/15-21	50.0	48.0	49.0	56.0	12.0	5.9	21.9	9.7	2.75	2.41	2.52	1.84	29.02		32.25	30.23	7.7	7.6
10/22-28	44.5	44.0	49.0		27.7	30.2	28.8	8.1	2.50	2.53	1.44	1.33	32.00	32.18	30.16	-	4.1	3.3
11/29-4																	4.3	
11/5-11	47.0	46.0	47.0	50.0	28.0	18.6	25.9	8.9	2.61	1.94	1.94	1.26	31.85	32.61	32.72	28.21	3.7	
11/12-18																	4.4	
11/19-25	46.4	46.4	46.0	41.9	28.4	27.5	29.8	10.8	2.54	2.84	2.31	2.09	31.92	32.45	32.29	26.44	3.7	

* Sequim Bay, Crescent Bay, Clallam Bay, Willapa Bay.

1/ Total amount of sunshine in hours daily average in calendar week.

APPENDIX Y

PRESENT STATUS OF THE PARALYTIC SHELLFISH POISON PROBLEM

By

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Paralytic shellfish poisoning has been recognized as a clinical entity for over a century, and the prevention of human intoxication by ingestion of toxic shellfish has been a problem to public health authorities for many years. Paralytic shellfish poison is of considerable concern because a fatal dose to man is only a few milligrams, which may be obtained from a single serving of highly toxic shellfish, and there is no known antidote. Prevention depends primarily on identification of poisonous shellfish by laboratory assay methods before they reach the consumer. Although such measures are necessary for protection of the public health, present day assay methods are laborious and costly, and require drastic control programs that cause severe operating problems for the shellfish industry. The butter clam industry in Alaska, for example, has been nearly destroyed because of the cost of surveillance. It is hoped that a systematic study of the shellfish poison problem will ease controls and make it possible to re-establish this industry.

A project is now in progress to (1) determine the feasibility of joining the paralytic shellfish molecule to other molecules to produce one or more conjugates having immunogenic properties, (2) develop a specific micro-assay method for identification of paralytic shellfish poison based on immunological reactions, and (3) lay the ground work for immunization of humans against the poison. The latter is considered only as a possible byproduct of this research since the prime objective is to obtain simpler and more sensitive tests for use in the field. A simple test could conceivably be used by the clam digger to check the toxicity of clams aboard a boat in Alaska and hence determine the acceptability of the product. A more sensitive test would be useful in studying the ecology of paralytic shellfish poison; such a test might be used to detect the poison in sea water and thus indirectly determine the presence or absence of Gonyaulax.

Assay Methods

In October 1958 the paper "Collaborative Study of the Bioassay for Paralytic Shellfish Poison" was presented at the annual meeting of the Association of Official Agricultural Chemists. At that meeting the method outlined in the paper was adopted by the Association as an official method of analysis, and it subsequently appeared in the February 1959 issue of J.A.O.A.C. The method also was subsequently published in the A.O.A.C.'s "Ninth Edition of Methods of Analysis."

A chemical assay for paralytic shellfish poison was studied collaboratively in 1959 and 1960. Although the method appears applicable to the assay of poison in clams, it was not successfully applied to mussels or oysters. It has not been adopted as an official method partly because of this limitation and partly because of the difficult and laborious procedure. Studies on assay methods, therefore, are continuing but with a slightly different approach.

Toxicity in Recent Years

As a result of an outbreak of human poisoning in Fanny Bay, Straits of Georgia, Vancouver, B.C., in November 1957, in which about 35 persons who had eaten oysters were involved, a testing program was initiated in the State of Washington and has been continued in the succeeding years. Since 1957 the toxicities of shellfish in both Vancouver (1) and the State of Washington (2) have seemingly fallen slightly each year. This year, for example, only one of many samples tested by the Food and Drug Directorate Canada, (1) was over 80 µg.

A similar pattern occurred on the East Coast, i.e., toxicities were quite high in 1957 when a few human poisoning cases occurred in New Brunswick, Canada, (3) but the toxicity of the shellfish declined slightly with each succeeding year. On the East Coast, however, very high toxicities were again encountered in 1961 (primarily in New Brunswick (3, 4) and Maine (5). In July, for example, toxicity in soft shell clams ran as high as 2,240 µg at Ross Island Thoroughfare in New Brunswick and 1112 µg at West Quoddy Bar in Maine; toxic shellfish were found as far south as Monhegan Island off the coast near Boothbay Harbor, Maine.

Fortunately, as a result of the high toxicities of shellfish in New Brunswick in 1957, Maine had started a testing program in July 1958 and was prepared to cope with the situation this year. Insofar as we know, no human illnesses occurred.

During the latter part of July 1961, Massachusetts (6) began testing shellfish for paralytic shellfish poison, and toxic shellfish were found in an area near Essex, Massachusetts. Although the toxicity of the shellfish was not high enough to be of concern, this area will bear watching again next year since it may be that the toxicity peak was missed because the testing program was started too late.

Other Studies

During investigation of an outbreak of human poisoning on the Pacific Coast in 1957, it became apparent that further studies on the ecology of paralytic shellfish poison were needed, particularly studies that might make it possible to predict when conditions are right for the occurrence of shellfish poison. If a system of prediction could be developed, the States might be relieved of some of the burden of maintaining an extensive and expensive testing program. As a result, in 1959 Dr. Albert Sparks of the College of Fisheries, University of Washington, applied for an NIH Grant to study this problem. He subsequently obtained such a grant, and the research has been in progress since early spring of 1961.

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APPENDIX Z

SANITARY SIGNIFICANCE OF FECAL COLIFORM ORGANISMS IN A SHELLFISH GROWING AREA. SANITARY SURVEY OF BURLEY LAGOON

By

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Introduction

As a part of the interstate cooperative study to determine the sanitary significance of fecal coliform organisms, the Shellfish Sanitation Laboratory cooperated with the Washington State Department of Health in conducting a survey of Burley Lagoon and its watershed. The lagoon is a marine estuary immediately adjacent to the Shellfish Laboratory. This area was chosen primarily because it is a representative shellfish growing area in the Pacific Northwest.

A comprehensive sanitary and bacteriological survey of a shellfish growing area and the adjacent watershed was made to determine sources of pollution and the relative quantities of coliform and fecal coliform organisms in the growing area and the tributary streams.

Description of the Marine Area and Watershed

Figure I shows the Burley Lagoon marine area and the contributory watershed. Burley Lagoon, a partially enclosed tidal basin at the northern end of Henderson Bay, is approximately two miles long and from one-third to one-half mile wide with the southern end partially enclosed. A channel approximately 400 feet wide connects the lagoon with the outer estuary. The whole tidal area, except the fresh water channels and two tidal basins, becomes dry at extreme low tides. Most of the lower two-thirds of the lagoon is utilized for the cultivation of the Pacific oyster, Crassostrea gigas.

Three main streams collect surface runoff from the surrounding country. Burley Creek is the largest and flows into the extreme north end of the tidal area. A tributary, known as Bear Creek, converges with Burley Creek approximately one-fourth mile above Burley Lagoon. The Burley Creek-Bear Creek watershed covers approxkmately ten square miles. Purdy Creek flows southwesterly and discharges into the southeast corner of Burley Lagoon. Purdy Creek watershed is approximately three square miles.

Sanitary Reconnaissance

A sanitary reconnaissance of the watershed was conducted by Mr. John Girard and Mr. Max Hayes of the Environmental Sanitation Section, Division of Engineering and Sanitation, Washington State Department of Health. Their observations are summarized as follows: Purdy Creek is a relatively clean stream throughout its length. The potential pollution-contributing population, both human and animal, is comparatively small and rather remote from the stream proper. Most of the water course is through heavily wooded areas and not readily accessible to domestic animals. In contrast, most of Burley Creek is directly exposed to a comparatively high potential contributing population composed primarily of cows. From the head of the stream to Station 18 (see Fig. 1) the contributing population is relatively small and this section is considered clean. In the present study none of the stations were found to be entirely free from pollution. The word "clean", therefore, is a relative term. Downstream from Station 18 much of the watershed is pastureland and the main stream and its tributaries are readily accessible to grazing animals. There is a small settlement on the lower reaches of the stream. All stations below 18 are considered to be polluted.

Bear Creek, which flows almost entirely through pastureland and is accessible to domestic animals, is polluted throughout its length. However, the tributary on which Station 19 is located flows through an uninhabited, wooded area and is relatively unpolluted.

In the estuarine area human sources of potential pollution exist between Station 3 on Purdy Creek and Station 8A on the bay. Because of strong indication that certain of these sources were discharging pollution intermittently during periods of high runoff Station 8A is considered polluted; Station 4 is polluted by the Burley Creek discharges and by sewerage discharges of human origin which may enter the bay near this station. Clean stream stations include Stations 3, 17, 18, and 19; all other stream stations are polluted. Polluted bay stations include Stations 4 and 8A, located near the discharges of Burley and Purdy Creeks respectively. The other bay stations, because of distance and dilution, are considered to be relatively clean.

Bacteriological Survey

Six sampling stations were established in the tidal area. and eleven on the tributary streams (Fig. 1). Locations on the tributary streams were determined by the results of the sanitary reconnaissance. Stations were established as near as possible to the source of the stream where potential pollution was limited and relatively remote, at strategic points downstream, and at the point of discharge into the tidal area. Sampling stations in the tidal area were located at points of maximum current flow.

Samples were collected from April, 1959, through July, 1960, during all four seasons under a variety of climatic and tidal conditions, and were submitted to the following bacteriological tests:

1. The multitube test for coliform organisms.
2. The E C liquid confirmatory test for fecal coliform organisms with incubation in a water bath at $44.5^{\circ}C \pm 0.2^{\circ}$.
3. The completed E C-IMViC test for classification of coliform types in the positive E C liquid confirmatory tubes. This test was applied to samples from key stations.

All tests were conducted in accordance with the methods in the APHA "Recommended Procedures for the Bacteriological Examination of Sea Water and Shellfish".

Results

Geometric mean, or median, coliform and fecal coliform MPNs from each station are shown in Table I. Except where noted, all are mean values taken from logarithmic probability plots; median values are used where this was necessitated by an excessive number of indeterminately low MPNs. With the exception of the value from Station 18, the lower coliform MPNs were from stations located near stream sources, relatively remote from pollution. The mean E C MPNs from stations remote from pollution (17, 18, 19) are considerably lower than those from other stream stations, which are located near or downstream from potential pollutional sources. In the bay area, the highest coliform and E C results are from Stations 4 and8A near the mouths of Burley Creek and Purdy Creek respectively. At all bay stations, the higher MPNs were obtained under low-ebbing tidal conditions. Considering the concomitant reduction in salinity, these results indicate that the coliform organisms in the bay have their sources in the fresh water streams.

Table 2 compares the coliform and E C MPNs of clean and polluted stations.

TABLE 2. COLIFORM AND E C MPNs FROM CLEAN AND POLLUTED STREAM AND BAY STATIONS - BURLEY LAGOON SURVEY

	Stream Stations				Bay Stations			
	Clean		Polluted		Clean		Polluted	
	MPN Per 100 ml.				MPN Per 100 ml.			
Percent Occurrence	Coliform	E C	Coliform	E C	Coliform	E C	Coliform	E C
10	29	2	410	110	1.8	--	1.8	--
50	250	20	2300	680	5.5	1.5	120	24
90	2200	210	13000	4000	110	24	7600	1100
Number of Samples	41		48		54		47	

The coliform and E C MPNs at the three percentile levels are from three clean and three polluted stream stations; bay results are based on combined MPNs from two clean and two polluted stations. The clean stream stations were 17, 18, and 19, and the polluted stations, 14, 13, and 1A. The latter stations are all located on Burley Creek and were selected because there was more information available on the contributing populations and their relationships to these stations. It should be pointed out that Station 1A is located below the confluence of Bear Creek and Burley Creek and would reflect, at least to a limited extent, influences from the former stream. Clean bay stations were Stations 7 and 8, and the polluted stations, 4 and 8A.

At all percentile levels, the coliform and EC MPNs from the polluted stream stations were significantly higher than those from clean stations. The relative increase in fecal coliforms compared to coliform types, from clean to polluted stream stations, was approximately 4 to 1, thus demonstrating a closer relationship between pollution and the fecal coliform types.

In order to establish the relationship between pollution and the incidence of fecal coliform types, the percentage of fecal coliforms in the total coliform population of clean and polluted stream and bay stations has been determined. This relationship, based on E C confirmatory/BGB confirmatory results, is shown in Table 3 and Fig. 2.

TABLE 3. RELATION OF CONFIRMED E C POSITIVE TO COLIFORM POSITIVE TUBES

Station Classification	No. of Coliform Positive Tubes	E Positive Tubes %
Stream Stations		
Polluted	796	81.5
Clean	575	51.1
Bay Stations		
Polluted		
All tides	267	63.3
High - flooding tides	65	36.9
Low - ebbing tides	169	70.4
Clean		
All tides	449	49.0
High - flooding tides	160	35.0
Low - ebbing tides	290	56.6

The direct correlation between pollution and the incidence of fecal coliform organisms is apparent from the fact that the percentage of fecal types contributing to the total coliform population is consistently higher from polluted than from clean stations. At the clean stream stations approximately 50 percent of the total coliform population were fecal types. In contrast, fecal types constituted approximately 82 percent of the coliform population from polluted stations. In the bay area the relationship between pollution and fecal types is best illustrated by considering the influence of tide on the incidence of fecal coliform organisms. At both clean and polluted stations the percentage of fecal types contributing to the total coliform population increased under conditions of low-ebbing tide, when the influence of fresh water was at or approaching a maximum. Because of proximity to fresh water sources this influence was much more marked at the polluted stations.

Coliform Types from E C Positive Tubes

The interim standard for eastern market oysters adopted at the 1958 Shellfish Sanitation Workshop included tolerances for fecal coliform organisms as enumerated by the E C test. In adopting the standard, it was assumed that the fecal coliform group, as isolated by the E C Method, consisted almost exclusively of E. coli type I. It was realized, however, that this assumption would have to be verified by a thorough evaluation of the E C test. The present report presents data derived from a study of:

(1) The validity of the E C method in enumerating E. coli type I.
(2) The incidence of other coliform types capable of producing gas in E C medium.
(3) The relative ability of the "fecal" coliforms and E. coli type I to reveal the presence of pollution.

E C gas positive tubes of stream and bay water samples from key stations were submitted to the E C - IMViC completed test. Stream samples were from Stations 17, 18, 19, 1A, 11, 13, and 3, and bay samples from Stations 4, 8A, 8 and the laboratory sea water supply.

TABLE 4. INCIDENCE OF COLIFORM TYPES ISOLATED FROM E C POSITIVE TUBES

	Stream Stations		Bay Stations	
	Number	Percent	Number	Percent
Tubes E C positive	1216		558	
E. coli type I	1102	90.6	507	91
E. coli type II	33	2.7	11	2.0
A. aerogenes type I	56	4.6	36	6.4
Intermediate types I & II	25	2.1	4	0.7

A summary of the data is presented in Table 4. Included under each category are the number of E C gas positive tubes examined, including each coliform type isolated, and the percentage incidence of each type.

Approximately 91 percent of the coliform types from both stream and bay stations were E. coli type I and approximately 93 percent were E. coli types I and II; A. Aerogenes type I accounted for 4.6 percent in stream waters and 6.4 percent in bay waters. The incidence of Intermediate types I & II was higher from stream stations; one station was responsible for almost all of the intermediates.

Productivity of E C Test

The relative productivity of the E C test at 24- and 48-hour incubation was investigated by an examination of more than 1800 E C gas positive tubes from samples of fresh water and salt water collected during the course of the survey. Table 5 summarizes these results.

TABLE 5. PRODUCTIVITY OF E C TEST AT 24 AND 48 HOUR INCUBATION

	No. of Tubes			Percent of all E C Positive Tubes		
	24 hrs.	48 hrs.	Total	24 hrs.	48 hrs.	Total
E C Positive	1787	23	1810	98.7	1.3	100
E. coli type I	1637	3	1640	90.4	0.2	90.6
Other coliform types	150	20	170	8.3	1.1	9.4

The data show that a very high percentage of the E C tubes produced gas within 24 hours of incubation; 98.7 percent of all the E C gas positive tubes were positive in 24 hours incubation. Thus, if time is important results can be obtained in 24 hours with a loss of only 1.3 percent of the fecal coliform organisms. Although data are not presented it has been observed that in most cases gas production is visible in the water bath within 8 hours of incubation.

A higher percentage of coliform types other than E. coli type I results with 48-hour incubation (Table 5); 0.2 percent of the E C positive tubes were found to contain E. coli type I, and 1.1 percent contained other coliform types.

Discussion

The evidence gathered from this study indicates a positive correlation between pollution and the incidence of fecal coliform organisms in both the fresh water streams and the estuarine area. Although in many instances fluctuations in the total coliform population correlated with changes in pollutional levels the entire coliform group was not as sensitive an index of pollution as the fecal coliform organisms. Where coliform and fecal coliform densities associated with pollution increased, the relative fecal coliform increases were definitely higher. Thus the more significant relationship appeared to be the percentage of coliform types that were E C positive. This has been demonstrated to be in clear correlation with the sanitary condition of a particular area.

Classification of coliform types isolated from the E C test positive tubes showed that approximately 90 percent were E. coli type I and approximately 93 percent were E. coli types I and II. With such a high degree of specificity for these fecal types, there appears little advantage in the more laborious and time-consuming procedure of classifying these types by the IMViC test.

Results of the study strongly indicate that the coliform organisms capable of growth at an elevated temperature had their source in fecal pollution; this supports the view that the fecal coliforms would be meaningful indicators of pollution in oysters as harvested. The use of fecal types has an additional advantage in that their enumeration can be accomplished in a relatively short time. Fecal coliform MPNs as enumerated by the E C method can often be made available within 36 hours and rarely require more than 48 hours.

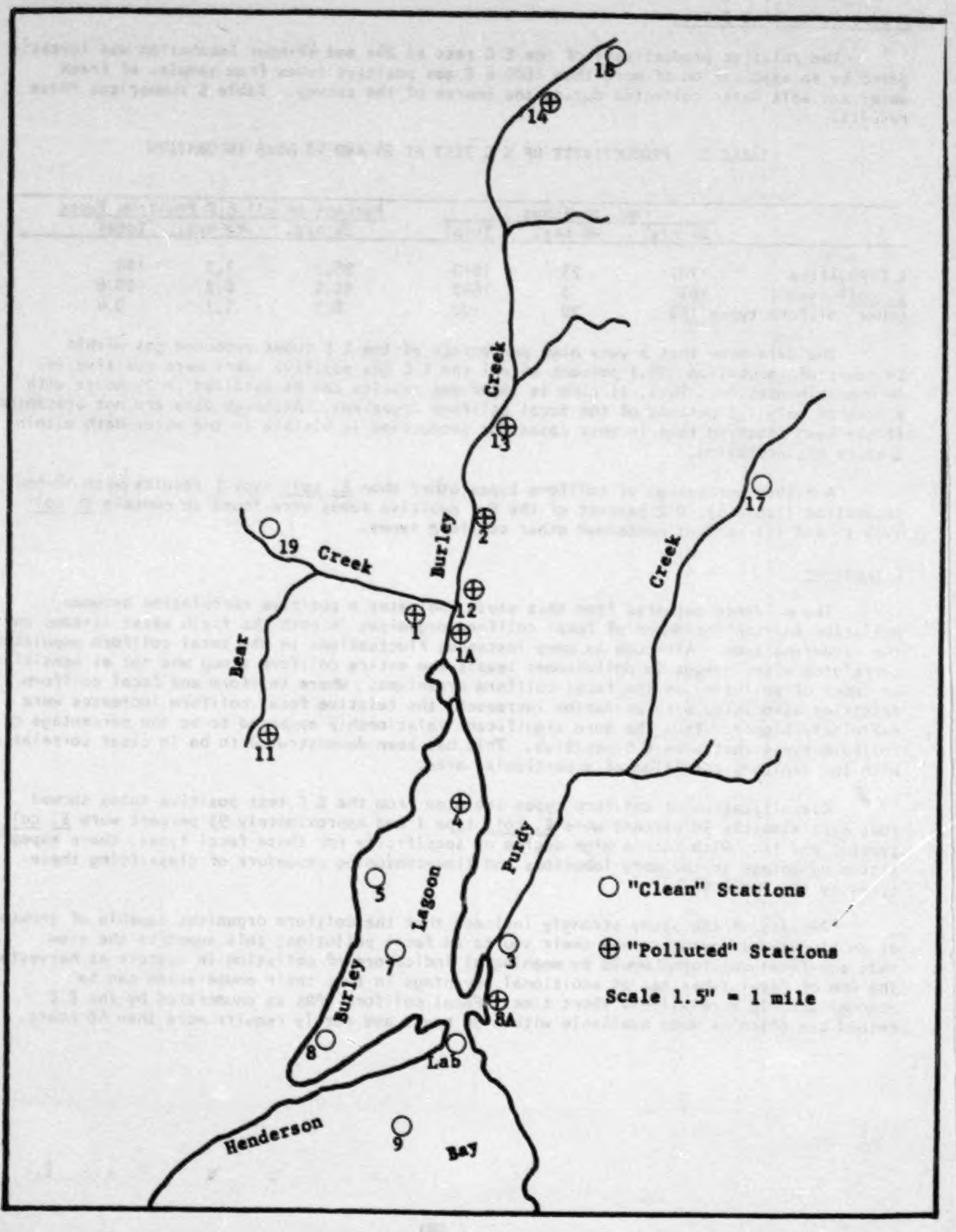
18
14
Creek
Burley
13
17
2
19
Creek
Creek
12
1
1A
Bear
11
4
Purdy
5
Lagoon
○ "Clean" Stations
7
3
⊕ "Polluted" Stations
Burley
8A
Scale 1.5" = 1 mile
8
Lab
Henderson
9
Bay

FIG. 1. BURLEY LAGOON WATERSHED AND MARINE AREA

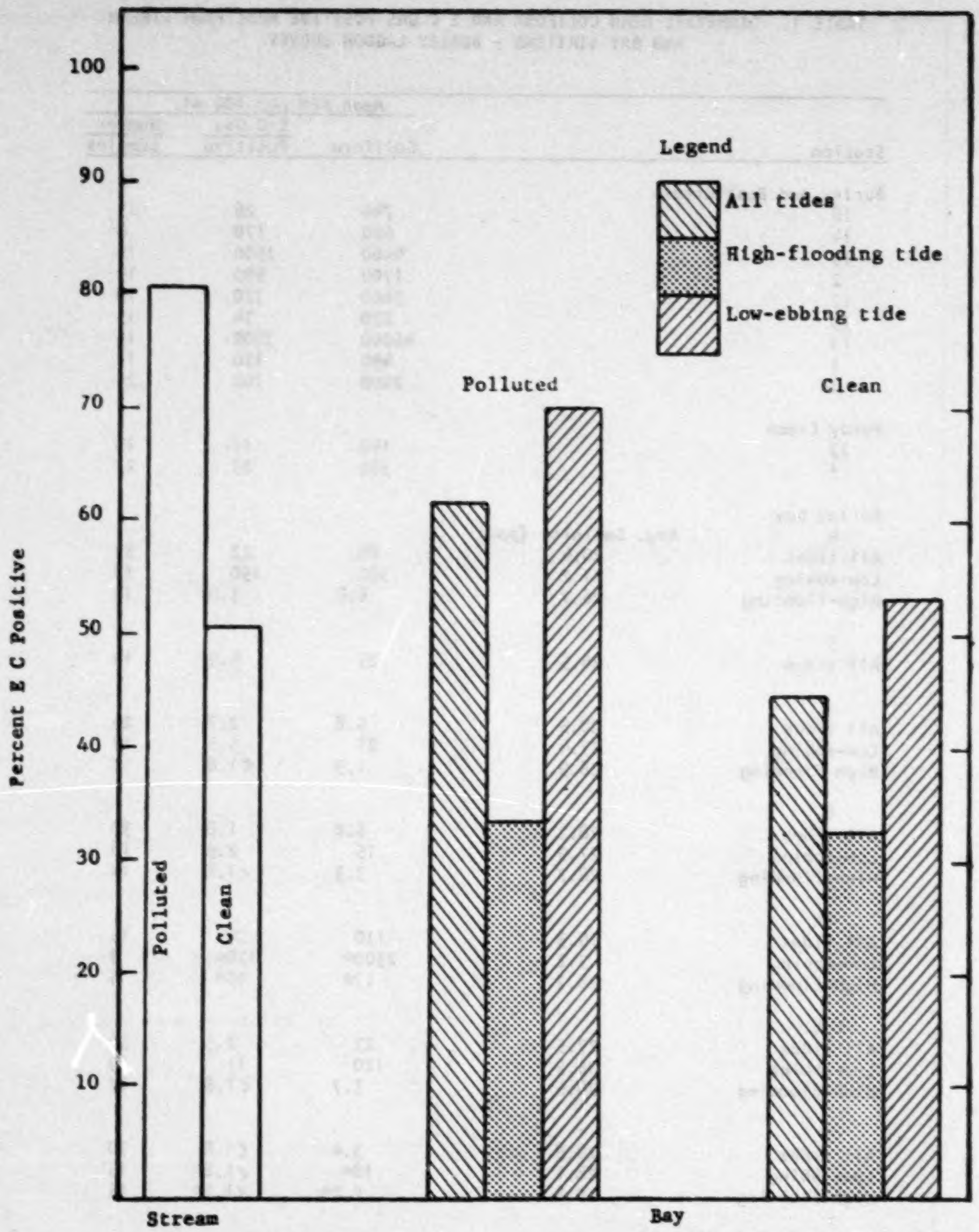

FIG. 2. RELATION OF CONFIRMED E C POSITIVE TO COLIFORM POSITIVE TUBES

TABLE 1. GEOMETRIC MEAN COLIFORM AND E C GAS POSITIVE MPNs FROM STREAM AND BAY STATIONS - BURLEY LAGOON SURVEY

		Mean MPN per 100 ml.		
Station		Coliform	E C Gas Positive	Number Samples
Burley and Bear Creeks				
18		740	26	13
14		620	170	8
13		4400	1500	15
2		1700	590	15
12		5600	720	10
19		220	34	13
11		46000	7800	15
1		490	120	17
1A		2400	700	25
Purdy Creek				
17		140	11	15
3		380	25	23
Burley Bay				
4	Avg. Salinity (ppt)			
All tides	20.4	88	22	32
Low-ebbing	11.2	380	150	18
High-flooding	26.7	8.8	3.0	14
5				
All tides	26.5	25	4.9	14
7				
All tides	28.5	5.8	2.7	24
Low-ebbing	27.4	21	5.3	12
High-flooding	28.9	1.9	<1.8	12
8				
All tides	28.2	6.0	1.8	30
Low-ebbing	27.9	15	2.9	16
High-flooding	28.7	3.3	<1.8	14
8A				
All tides	15.3	110	29	15
Low-ebbing	3.2	2300*	170*	9
High-flooding	28.1	17*	15*	6
Lab.				
All tides	27.5	27	2.5	36
Low-ebbing	26.5	120	11	19
High-flooding	28.6	3.7	<1.8	17
9				
All tides	28.5	3.4	<1.8	30
Low-ebbing	28.1	18*	<1.8*	15
High-flooding	28.8	2.0*	<1.8*	15

* Median values

APPENDIX AA

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★ U. S. GOVERNMENT PRINTING OFFICE : 1962 O - 655625

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